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# NORTH AMERICAN PARASITIC COPEPODS.

## PART 9.

## (THE LERNÆOPODIDÆ.)

B 485159

BY

CHARLES BRANCH WILSON.

Dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the Degree of Doctor of Philosophy: Baltimore, 1910.

(Reprinted from Proceedings U. S. National Museum
Vol. 39, No. 1783)

WASHINGTON
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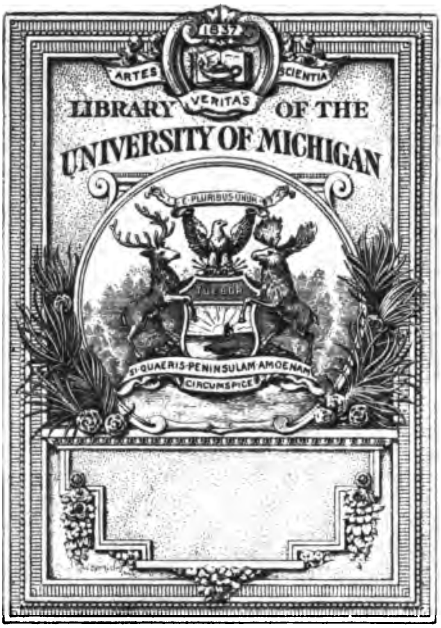
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# VITA.

Charles Branch Wilson was born October 20, 1861, at Exeter, Maine. He graduated from Colby College in 1881 with the degree of A. B.; was a postgraduate student and tutor in Botany at Colby from 1881 to 1884, receiving the degree of A. M. in the latter year, and was given the honorary degree of Sc. D. in 1908.

From 1884 to 1891 he was a private tutor; from 1891 to 1894 Professor of Natural Sciences at the State Normal School at Gorham, Maine; from 1894 to 1896 a postgraduate student and student assistant in Biology at Johns Hopkins University; since 1896 Professor of Biology at the State Normal School at Westfield, Massachusetts; a postgraduate student and fellow by courtesy at Johns Hopkins University during the school year 1909–1910.

During the summer months of 1895 and 1896 he was director of a summer school in Biology at Harpswell, Maine; in 1897 a member of the Johns Hopkins Biological Expedition to Jamaica; in 1898 and 1899 a research worker in the Tufts College Biological Laboratory at Harpswell, Maine; since 1899 a temporary assistant of the U. S. Bureau of Fisheries. In this latter work the summers of 1900 to 1904 were spent at Woods Hole, Massachusetts, 1905 at Beaufort, North Carolina, and 1906 at Lake Maxinkuckee, Indiana, in investigating the Copepod parasites of our food fishes. The summer of 1907 was spent on the Mississippi, Ohio, and Tennessee rivers as a member of the Fresh-water Mussel Commission; 1908 on the Maumee and Wabash rivers in Indiana and Ohio, and 1909 on the Kankakee river in Indiana and Illinois, investigating the life and habits of fresh-water mussels.

During the summer of 1910 he was again a member of the Johns Hopkins Biological Expedition to Jamaica.

In 1901 the collection of Parasitic Copepods belonging to the U. S. National Museum was placed in his hands for study and determination. The nine papers belonging to the present series, which are

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enumerated on the first page of this thesis, are the outcome of this work. Beside these there have also been published the following papers.

1897. The Wrinkling of Frog's Eggs during Segmentation, Amer. Nat., 1896, pp. 761 to 773, pls. XIII and XIV.

1898. Activities of Mesenchyme in Certain Larvae, Zoological Bulletin, vol. 2, no. 1, pp. 15–22, 4 text figs.

1899. The Habits and Early Development of Cerebratulus lacteus Verrill, Quart. Jour. Micr. Sci., n. s, vol. 43, pp. 97–198, pls. 9–11.

1900. Our North American Echiurids, Biol. Bull., vol. 1, pp. 163–178, pl. 1.

1904. The Fish Parasites of the Genus Argulus found in the Woods Hole Region, Bull. Bureau of Fisheries, vol. 24, pp. 115–131, 31 text figures.

1905. New Species of Parasitic Copepods from the Massachusetts Coast, Proc. Biol. Soc. Washington, vol. 18, pp. 127–132.

1906. On Some Parasitic Copepoda, Ceylon Pearl Oyster Report, pt. 5, supplementary report 34, pp. 189–210, pls. 1–5.

1909. Dragonflies of the Mississippi Valley, Proc. U. S. Nat. Mus., vol. 37, pp. 653–671.

# NORTH AMERICAN PARASITIC COPEPODS.—PART 9. THE LERNÆOPODIDÆ.

By Charles Branch Wilson,
*Department of Biology, State Normal School, Westfield, Massachusetts.*

## DEVELOPMENT OF ACHTHERES AMBLOPLITIS KELLICOTT.

### INTRODUCTORY.

The present paper is the ninth[a] in the series dealing with the North American Parasitic Copepods. It takes up the development of a species which is the American representative of the European *Achtheres percarum* Nordmann, and which may serve as the type of the family Lernæopodidæ.

This American species is very common upon the gill arches of the rock bass or red-eye, *Ambloplites rupestris*, and is occasionally found upon other species of the Centrarchidæ.

At the present time it has never been obtained from the American perch, or from any other of the Percidæ.

It lives fastened to the inner surface of the gill arches and easily escapes detection, especially in the larval stages, by concealment among the large teeth which cover that portion of the arches, and by being covered with the slime that envelops the whole of the gill surfaces.

The surest method of detecting its presence and the one by means of which all the larval stages here described were discovered, is to cut out the gills carefully, separate each arch from the others, and

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[a] The other eight papers are as follows: 1. The Argulidæ, Proc. U. S. Nat. Mus., vol. 25, pp. 635–742, pls. 8–27. 2. Descriptions of Argulidæ, idem, vol. 27, pp. 627–655, 38 text figures. 3. The Caliginæ, idem, vol. 28, pp. 479–672, pls. 5–29. 4. The Trebinæ and Euryphorinæ, idem, vol. 31, pp. 669–720, pls. 15–20. 5. Additional Notes on the Argulidæ, idem, vol. 32, pp. 411–424, pls. 29–32. 6. The Pandarinæ and Cecropinæ, idem, vol. 33, pp. 323–490, pls. 17–43. 7. New species of Caliginæ, idem, vol. 33, pp. 593–627, pls. 49–56. 8. Parasitic Copepods from the Pacific Coast, idem, vol. 35, pp. 431–481, pls. 66–83.

Proceedings U. S. National Museum, Vol. 39—No. 1783.

float it out in water under a dissecting microscope. The buoyant power of the water will lift the larva or adult into view above the slime and teeth, particularly if the dish be agitated a little.

The living material upon which the following observations were made was obtained at Lake Maxinkuckee, Indiana, during the summers of 1906, 1908, and 1909, while the author was in the employ of the U. S. Bureau of Fisheries. For this valuable opportunity acknowledgment is gratefully made to the Hon. George M. Bowers, U. S. Commissioner of Fish and Fisheries. The serial sections and the study upon them were made in the biological laboratory of Johns Hopkins University, and the sincere thanks of the author are due to Dr. E. A. Andrews for many valuable suggestions and corrections.

## METHODS.

For external study, including the mouth-parts and other appendages, a mixture of 95 per cent alcohol and 5 per cent formalin in equal parts was found to be the most satisfactory preservative. Specimens kept in this mixture for three years have retained their anatomical form and structure perfectly, and have become neither unduly hard nor brittle.

For the histological work the material was preserved in alcoholic corrosive-acetic, the corrosive being removed immediately after fixation with iodine.

The specimens to be sectioned were first stained in bulk with Delafield's hæmatoxylin, and after clearing were counterstained with eosin in 95 per cent alcohol.

These methods were found to give excellent results both in fixation of the tissues and in differential staining.

## HISTORICAL.

The genus *Achtheres* was established by Nordmann in 1832, with the type species *Achtheres percarum*, found in great abundance on the gill arches of the common European perch, *Perca fluviatilis*. Nordmann not only described the adults of both sexes minutely, but he also gave a good account of the breeding habits and the development up to the first copepodid stage. In the same paper he established another genus, *Tracheliastes*, closely related to *Achtheres*. He did not obtain the male of his type-species, *Tracheliastes polycolpus*, but did secure some newly hatched larvæ, whose development he also followed up to the first copepodid stage. In a third new genus, *Basanistes*, with the type-species *huchonis* described at the same time, he was not so fortunate, and no developmental stages are mentioned.

Three years later (1835), however, Kollar reinvestigated this third species and included with the adults a good description and figures of the first copepodid larva.

Here the development of the Lernæopodidæ rested for nearly thirty years until Claus in 1862 filled in one of the missing stages in the life history of *Achtheres percarum*. But Claus himself stated that he was unable to finish his investigations, and many gaps were still left in our knowledge of this crustacean family.

In 1870 Édouard Van Beneden gave as the fourth paper in his researches on the embryogeny of the crustacea what might be called a mosaic development of the Lernæopodidæ.

His series of stages, which began with the segmentation of the egg and closed with this same first copepodid larva, were selected from the genera *Anchorella*, *Lernæopoda*, *Brachiella*, and *Hessia*.[a]

Olsson in 1877 published the figure of a larva of *Achtheres percarum*, but gave an extremely meager description, while the figure itself was so small as to show no details.

Finally Vejdovský in the same year (1877) worked over again the anatomy and development of *Tracheliastes polycolpus*, but like Nordmann and Kollar he followed the metamorphosis only to the first copepodid stage. His description and figures of the development inside the egg, however, are the most complete and the best that have ever been published.

These five papers (beside Olsson's single figure) comprise practically all that has appeared upon the development of this family, the Lernæopodidæ, up to the present time.

The reason why so many of them stop with the first copepodid larva is readily understood when it is recalled that this is the only free swimming stage, and is therefore the one during which the larva seeks out its host. Even Claus, who is the only one to describe any of the subsequent stages, was forced to be content with female larvæ 2 mm. long (really adults) and a male larva that was practically fully developed. But he described both of these in considerable detail and thus furnished an important contribution to their life history.

Claus gave it as theoretically probable that this first copepodid stage, at which all the accounts stop, passed at the next molt into an attached form in which the number of body segments and appendages was not increased, but the frontal filament was put into operation for attachment to the host, the mandibles were inclosed in the proboscis, and the setæ of the swimming legs and the furca were degenerated from lack of use. He was not able to find such a form, but it has been discovered in connection with the present work, and while it does not conform in all details to what he prophesied, it shows a remarkably accurate conception on his part of its general features. But his actual observations were made upon larvæ and adults entirely, that is, without the aid of dissection or serial sections, and the same may be said of all the other investigators, whether working with eggs, larvæ, or adult stages. Since all of these are more or less opaque it is not surprising that some mistakes were made in observations. The wonder rather is that so many of these observations of the exterior are substantiated by sectioning the different stages.

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[a] This was a new genus named for the first and as it has proved the last time, but not described or figured, except in one or two embryonal stages.

### COMPARISON WITH OTHER PARASITIC SPECIES.

On comparing carefully and in detail the metamorphoses which the different species exhibit, we may separate the parasitic copepods into five groups according to the relative lengths of the free-swimming and parasitic periods:

I. Those whose larvæ are free swimmers during their entire development and become parasites only upon reaching the sexually mature adult condition.

The Ergasilidæ are examples of this group, and in the genus *Ergasilus* the males never become parasites at all but remain free during life.

II. Those in which the earlier larval stages and the mature adult are free-swimming, while the intervening stages are parasitic and sometimes degenerate.

Here belong the Monstrillidæ and the males of certain genera among the Ascidicolidæ. The males and often the females of some of the Caligidæ might also be well placed here, since the mature adults are frequently captured swimming at the surface, and only attach themselves temporarily to fish in order to obtain food.

III. Those whose larvæ are free swimming during early development, passing through typical nauplius, metanauplius, and copepodid stages, and then seeking a host upon which to become parasitic during the remainder of life.

The stage at which the change is made varies considerably, as does also the life history subsequent to it. In some of the Ascidicolidæ the larvæ remain free-swimming until the second copepodid stage, as in *Enterognathus*. In the Pandarinæ, Cecropinæ and Chondracanthidæ they become parasitic at the beginning of the first copepodid stage. In the latter family they are transformed at the next molt into the adult, the other copepodid stages being suppressed.

IV. Those in which the early stages are often passed inside the egg, while later free-swimming stages alternate with others which are both parasitic and degenerate.

The Lernaeidæ are examples of this group; the larvæ are free-swimming until the first copepodid stage, then become parasitic and degenerate into a pupal form in which the power of movement is lost. Later they regain this power, leave their host, and swim about freely while a union of the sexes takes place. The male develops no farther but the female seeks a second host and on it undergoes a new degeneration, even greater than the first.

V. Those in which the first copepodid stage alone is free-swimming; all the larval development previous to this is passed inside the egg, while subsequent to it the copepod is a fixed parasite, usually showing degeneration.

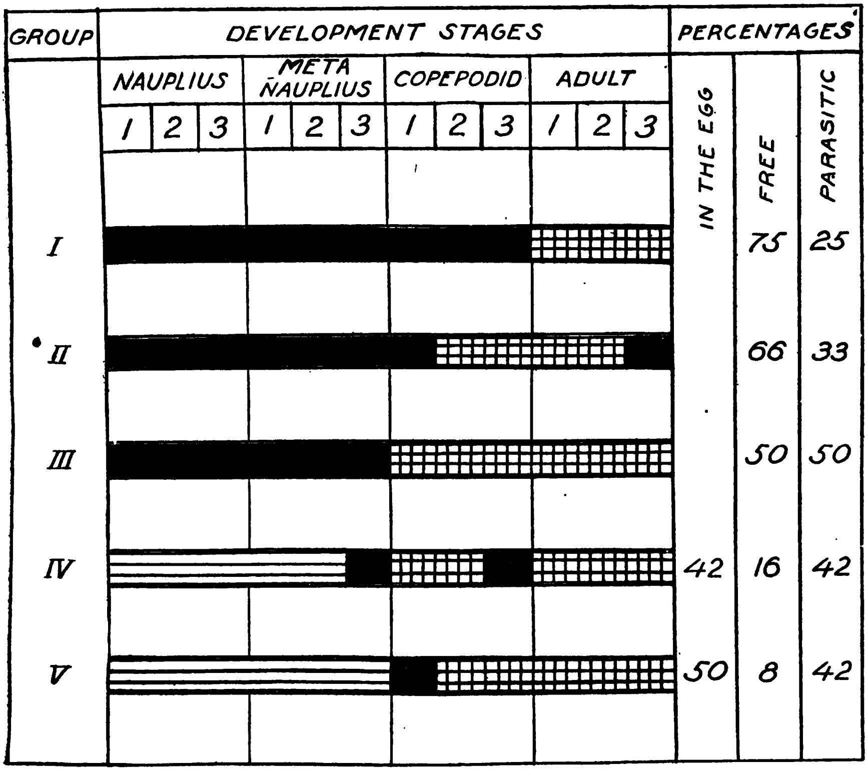

TABLE SHOWING COMPARATIVE TIME SPENT IN THE EGG (HORIZONTAL LINES), AS FREE SWIMMERS (BLACK), AND AS PARASITES (SQUARES), BY THE DEVELOPING LARVÆ OF TYPICAL GENERA FROM EACH OF THE FIVE GROUPS MENTIONED.

Here belong the Choniostomatidæ and the Lernæopodidæ. In the latter family, according to Claus and others, some species hatch in an advanced metanauplius stage, all ready for the molt into the first copepodid stage. This molt consequently takes place in a remarkably short time, from ten minutes to an hour. In other species, as observed by Kellicott and the present author, the larvæ hatch directly into the first copepodid stage and seek a host at once.

The details of this extraordinary shortening of the free larval period have never been brought out clearly, and that is the aim of the following account.

## THE EGG PREVIOUS TO DEVELOPMENT.

### FORMATION OF THE EGG.

Claus (1862) was the first to call attention to the long threads or filaments which are connected with the older oöcytes inside the ovary of *Achtheres.* Beneden (1870) found the same condition in *Anchorella* and *Lernæopoda,* while Kerschner (1879) and Giesbrecht (1882) discovered it in *Congericola, Doropygus, and Notopterophorus.* The same filaments are found in connection with the eggs of *Achtheres ambloplitis.* On being magnified (fig. 1) these filaments are seen to be composed of cells, usually discoidal in shape and packed with their flat surfaces together like rows of coins. A cross section of a uterine process in which the eggs are partly matured shows one of these filaments attached to each egg and packed more or less closely against the surface of the latter. Owing to the crowding of the eggs in these uterine processes, the threads or filaments are often coiled into a sort of ball, as seen at *b* in fig. 1. It will be noticed that the cells at one end of the filament are greatly reduced in size, and Korschelt and Heider suggest that the formation of new cell material possibly takes place here. At the opposite end of the thread is the oöcyte itself, abruptly and enormously (compared with the cell filament) swollen in size.

This increase in size is due wholly to the absorption of food material or yolk globules by the oöcyte, the difference in composition being clearly brought out by staining.

The entire substance of the cell filament takes a deep blue color in hæmatoxylin, while the oöcyte, except the nucleus, takes none whatever, but stains a deep red in eosin.

According to Giesbrecht, the rows of cells loosen themselves from the epithelium of the ovary in order to connect with the oöcytes. But whatever their origin, it is certain that they are no longer connected with any part of the ovary in the present species, and have been pushed far out into the uterine processes. As soon as the terminal cell has become fully developed into the oöcyte it separates from the cell filament, and the cell, which then becomes terminal in the filament, develops in its turn. And thus the process continues until all the cells in the filament have been successively formed into eggs.

As Beneden pointed out, the difference in egg development between this family of Lernæopodidæ and the Caligidæ lies in the fact that the latter possess but a single ovarian filament, in which a series of cells develops simultaneously.

Here in *Achtheres* (the Lernæopodidæ), on the contrary, there are many of these ovarian cell filaments, and in each of them the terminal cells develop successively, one after the other. A similar method obtains in the Ergasilidæ, whose eggs mature successively and are pushed out into the external strings in bunches, a few at a time.

### STRUCTURE OF THE EGG.

Beneden states that he was never able to distinguish any membrane around the ovarian egg of *Anchorella* or *Lernæopoda*, but that the greater density of the protoplasm near the periphery of the egg preserved its spheroidal form.

In *Achtheres ambloplitis* a vitelline membrane is distinctly visible around the enlarged oöcytes, even before they separate from the cell filaments (fig. 2). This membrane is very thin and structureless; inside of it the entire substance of the egg is made up of yolk globules evenly and universally distributed throughout a matrix of fine protoplasm. These globules differ only slightly in size and shape, being usually somewhat ovate or ellipsoidal and occasionally flattened by contact with one another. They are homogeneous in structure, but some of them stain more deeply than others.

Scattered about through the egg are numerous large vacuoles, varying from one to four times the diameter of the yolk globules. In the sections they are entirely empty, save for the intrusion of an occasional yolk globule, but in the living egg are probably filled with a liquid which has been withdrawn by the preservatives. The vacuoles nearest the periphery are usually the smallest, and they increase in size toward the center of the egg. The egg nucleus is about the size of the largest vacuoles and is slightly eccentric, away from the point of attachment of the cell filament. It is approximately spherical and is surrounded by a well-defined membrane. It contains a single spherical nucleolus of small size and situated close to the periphery, and numerous chromatin granules, also gathered near the outer surface of the nucleus. The cytoplasm just inside the vitelline membrane is denser than elsewhere, and particularly in the younger oöcytes takes a bluish tinge in hæmatoxylin. In the matured oöcytes this is scarcely visible, but the increased density and the fineness of the granules are very distinct.

As the eggs pass down through the oviduct they are each fertilized at the opening of the sperm receptacle and are covered with a layer of the cement substance at the opening of the duct of the cement gland just before they pass out into the external sacks. This cement forms a thick outer egg membrane or shell, separated a little from the vitelline membrane and stiffening into a strong and tough covering.

Beneden called the vitelline membrane the chorion and this shell membrane the exochorion. The egg sack itself is made of the same material, only thicker and tougher. Inside of it the eggs lie freely together and are not separated one from another by secondary walls as in the Caligidæ. But the same free space around each egg, necessary to secure the requisite supply of oxygen for the developing embryo, is obtained by the separation of the two membranes covering the egg. The stiff outer membrane, which ordinarily fuses with the vitelline membrane into a honeycomb substance, here remains separate, and swelling a little after entering the external sack, maintains a narrow space for aeration around the egg. The loose manner in which the eggs are packed inside the external sacks favors such a method of aeration. Instead of being arranged in a single row pressed tightly together and strongly flattened at right angles to the long axis of the sack, they are thrown in loosely without any definite arrangement. The former conditions which prevail in the Caligidæ, Dichelestiidæ, and Lernæidæ necessitate the presence of secondary partitions to prevent the embryos from being smothered. But the latter condition, which is common to free swimming forms and to the Ergasilidæ and Chondracanthidæ as well as the present family, can dispense with these partitions, the stiff shell membrane serving amply to keep the eggs properly aerated.

## EMBRYONIC DEVELOPMENT WITHIN THE EGG.

### ARRANGEMENT OF EMBRYOS.

Owing to the lack of any definite arrangement of the eggs within the external sacks, there is a corresponding absence of regularity in the position of the embryos.

In the Caligidæ the germinal area is found in the center of the proximal side of each of the flattened eggs, and the longitudinal axes of the developing embryos are closely parallel.

The only general statement that can be made with reference to the present species is that development usually begins at that pole of the egg which is diametrically farthest away from the external surface of the egg sack. This point afterwards becomes the posterior end of the enbryo so that, when fully developed as well as during development, the larvæ lie in the egg sacks with their heads toward its outer surface; that is, they are arranged radially.

Further than this there seems to be nothing definite, since the germinal area spreads with equal impartiality over any of the various surfaces toward the egg sack. And in those eggs which lie near the center of the sack and entirely surrounded by other eggs, the point where development begins may be turned in any direction indiscriminately.

### CLEAVAGE STAGES.

Segmentation is purely superficial, proceeding gradually from the point just mentioned over the whole surface of the egg. Beneden has stated that in *Anchorella*, *Lernæopoda*, and *Brachiella*, which are near relatives of *Achtheres*, the cleavage is discoidal. This means that a single blastoderm cell originally becomes entirely separated from the food yolk, and that this one cell by subsequent division yields the whole blastoderm. On the other hand Korschelt and Heider have suggested that the increase of blastoderm in these cases is really due to the accession of new elements from the interior of the egg. In the present species we may clearly distinguish a combination of these two methods.

The first cleavage nucleus of the egg divides twice or more, and with some of the cytoplasmic elements found within the yolk migrates to a definite point at the surface (fig. 3).

In the majority of cases, as already stated, this point is diametrically opposite the wall of the egg sack, and, what is more important, it becomes the posterior end of the embryo.

Schimkevitch (1896) has stated that in *Tracheliastes* this migration is toward the wall of the egg sack and toward the places where the eggs touch one another. And he adds in a footnote that the direction of migration is not determined by oxygenotaxis, since in this case it would always be toward the wall of the egg sack. The present species offers still more lucid proof of this statement since the migration is directly away from the outer egg sack. But it must be kept in mind that this eventually brings the anterior or head end of the embryo nearest the outer wall where there is the best oxygenation.

At the time of migration small particles appear between the yolk globules, scattered about rather uniformly. These take a blue stain very readily and are therefore different in nature from the yolk. They look exactly like the particles of chromatin in the original cleavage nucleus, and like those which subsequently appear in the blastoderm cells, but there is no means at present of definitely proving their nature. They are not found in the unfertilized egg and they entirely disappear by the time the blastoderm is completed. They are the small black dots seen in figs. 3 to 7.

When these migrated materials reach the surface they are there transformed into blastoderm cells by an accumulation of the cytoplasmic material around nuclear centers. At first a single large cell is formed (fig. 4), ovoid in shape, its long diameter one-fourth that of the egg. Around this cell is a considerable mass of surplus material, similar in nature and containing one or more nuclear centers.

By the differentiation produced in double staining this material may be followed far down into the yolk of the egg from whence it is migrating. The large cell, however, in this as well as in subsequent stages, is entirely separate from the yolk and simply rests upon the surface of the egg.

The cytoplasmic material goes on collecting around a second nuclear center and shortly we find two large cells of practically the same size (fig. 5).[a] Again these are surrounded by considerable surplus material and now there are several accessory nuclear centers. Again also we can follow the migrating material for some distance into the yolk. We may call this a two-cell stage, but we must remember that it is not such in the sense that the two cells are the halves of the original one. They have rather been built up independently of each other, and are each formed of separate material that has migrated out of the yolk.

This same accumulation of material around nuclear centers goes on until there are four or five of these large cells, of about the same size as the original one. Then, while the same process continues, a segmentation of these large cells also begins, and we find eggs with from 12 to 16 medium-sized cells, only half their former diameter (fig. 6).

There is still much surplus material around them and many small nuclear centers, but the migrating material can not be followed as readily into the yolk.

The amount of migrating material and the rapidity with which it comes out from the egg would seem to exert a controlling influence upon the kind of segmentation which results.

If the cytoplasm and nuclear material emerge all at once, the former gathers around the latter in a single mass, which by subsequent division forms the blastoderm. This is true discoidal segmentation. But if the substances migrate more slowly, those which reach the surface first have time to accumulate while the others are still emerging. The result is not a single cell but several such cells of nearly the same size, as in the present instance. Moreover the removal of the last of this material from between the yolk globules is accomplished more slowly than at first, either because, coming from the center of the egg, it had a longer distance to travel, or because the actual rate of emergence has diminished, or possibly both of these combined. The blastoderm cells first formed thus have plenty of time to segment before the material is all out of the egg. And since there is not as much of the migrating material now as there was at first, the size of the accessory cells which it forms and adds to the blastoderm is about proportional to the size of the cells in the latter. Since these accessory cells become a part of the blastoderm and enter into the formation of the embryo, this can not be regarded as discoidal segmentation.

### BLASTULA.

The two processes of aggregation and cell division go on simultaneously until the blastoderm has spread superficially over the entire yolk. In the intermediate stage represented in fig. 7 it can be seen that the blastoderm cells have appreciably diminished in size, and it is now very difficult to trace any of the migrating material within the yolk. The disk remains thicker over the point where the cells originally appeared, but elsewhere is only a single cell in thickness. When it is finally completed (fig. 8) its cells are very much reduced in size, and no accessory material can be seen around them; it has all entered into the structure of the blastoderm. As soon as this has been accomplished the cells composing the blastoderm secrete a cuticular membrane in addition to the vitelline and shell membranes. This has been designated as the blastodermic cuticle, and according to Korschelt and Heider its appearance can only be explained by regarding it as a sort of ecdysis or molt, carried back to an early embryonic period. The formation of this cuticle thus constitutes the first molt of the *Achtheres* embryo. Such a cuticle is common among the Malacostraca, but in the copepods is probably confined to those cases like the present, in which many of the stages of development are shifted back into embryonic life.

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[a] In the figure the right cell is cut at a different level and so appears smaller than the left one; in the second section following it is fully the size of the latter.

### FORMATION OF THE EMBRYO.

Not until the blastoderm has entirely surrounded the yolk is there any differentiation in it to indicate the position of the larval appendages. The first change is a considerable thickening of the cellular layer which is to become the ventral surface of the embryo (the "Keimstreif").

The multiplication of cells is especially rapid along the axes of the future appendages and builds up there a series of lobes or pads which constitute the "Kopfplatten" of the German embryologists. Ordinarily there are three pairs which have come to be known as the nauplius appendages, which appear simultaneously and develop into the locomotor organs of the first larval stage. These three develop together into certain typical forms without reference to what may be the shape and function of the matured organ which they represent. For instance, the third pair are usually identical, whether they are to become the gnawing mandibles of the free-swimmers or the piercing mandibles of the parasites. Moreover, the three pairs develop and have served their temporary function before the other appendages appear.

In the formation of the nauplius of the present species we notice several radical departures from this established type.

When a longitudinal section of the developing embryo is examined it reveals nuclear centers not only for the first three appendages but also for the other mouth-parts and the first two pairs of swimming legs (fig. 9). These centers all appear simultaneously, but those of the first two pairs develop much faster than the rest. And they form typical nauplius appendages before any of the others have become externally visible. The third pair never become nauplius appendages but appear as rudimentary mandibles, without showing any traces of division into the usual two rami, of segmentation, or of the ordinary nauplius armature of plumose setæ. All the other appendages and the rudiments of an upper and an under lip appear at the same time with the third pair, and are developed directly into the forms found in the metanauplius. It will be recalled that it was the appearance of the two posterior pairs of mouth-parts side by side in *Achtheres percarum* that induced Claus to first put forward the idea that they developed as the exopod and endopod of the same appendage. Claus himself corrected this idea in almost the last paper he published, having found the two appendages to be distinct on the long-bodied larvæ of certain marine copepods. Moreover he discovered that the posterior pair originated behind the groove separating the head from the thorax, and were therefore thoracic, while the anterior pair were cephalic. The same evidence can now be presented from the very genus upon which Claus originally worked. If fig. 9 be examined again it will be seen that the groove representing the boundary between the head and thorax appears between the last two pairs of mouth-parts, thus making the posterior pair thoracic and the anterior pair cephalic. This groove only shows in sections and between the nuclear centers or very beginnings of the appendages. It also disappears before the appendages become visible externally, but it is very distinct while it lasts and its significance can not be mistaken.

The posterior body develops along with the appendages, and by the time the first and second antennæ are formed we find a thorax and abdomen very similar to that which ordinarily appears in a metanauplius larva. The balancers, which are so typical of nauplius larvæ, never appear at all; in fact, the posterior body seems to start in the metanauplius form from the very beginning and never displays any of the nauplius characters. With these few words of explanation as to the formation of the embryo we shall be the better prepared to understand the remaining development inside of the egg.

### NAUPLIUS-METANAUPLIUS STAGE.

In many of the parasitic as well as all of the free-swimming copepods there is a long line of nauplius and metanauplius stages, distinctly separated from one another by a throwing off of the old skin and the formation of a new one.

In the present species there is a complete fusion of all these stages, with nothing left to indicate their presence except the formation of a single nauplius cuticle.

This larval integument, which is the fourth in chronological order, is formed about the middle of the fused stage and may fairly be taken to represent the close of the nauplius and the beginning of the metanauplius stage. It is therefore the second molt of the developing larva. But the creature inside of this larval integument is very different from an ordinary nauplius.

*Body form of nauplius.*—If some of the larvæ be dissected out of their surrounding envelopes at this stage and mounted under a gentle pressure, they present in dorsal view the general features seen in fig. 10. The body is ovate in outline, the larger end being anterior, the two diameters in the proportion of 7 to 11, and the greatest width about one-third the distance from the anterior end. Only the first two pairs of appendages are visible, corresponding to the first and second antennæ, and projecting nearly their length beyond the margin of the body. Around these two pairs of appendages the nauplius cuticle forms lobes or pockets, as can be seen in fig. 12. The anterior pair, the first antennæ, are one-jointed and uniramose, of the same diameter throughout, and each terminating in two long plumose setæ.

The second antennæ are biramose, the exopod five-jointed, with each joint terminating in a long plumose seta, the endopod indistinctly two-jointed and terminated by two plumose setæ.

The body itself is transparent throughout and shows no trace of mouth, stomach, or intestine. The anterior portion is made up of fine granular protoplasm, from which is to be derived the material for the formation of the mouth-parts, the beginnings of the nervous system and sense organs, the larval muscles, and the large frontal gland soon to be described.

Along either side, parallel with the margin and close to the dorsal surface, is a broad ribbon-like band of striated muscle fibers, the first beginning of the muscular system which is to operate the swimming legs.

The extreme posterior part of the body is curled over ventrally beneath the anterior portion. In a ventral view (fig. 11) it may be seen to consist of a broad and spatulate abdomen, already well differentiated from the cephalothorax, and made up like the anterior part of the body of fine grained protoplasm, thickly studded with nuclei. The mandibles and upper lip have not yet appeared externally save as a slight transverse ridge with a knob at either end of it, just behind the bases of the second antennæ. The posterior part of the body therefore is like the metanauplius, while the anterior portion is just as typically nauplius, and the two stages are combined in one embryo.

The entire body is uncolored save for three isolated patches of dark-brown pigment. One of these is on the dorsal surface in the center of the widest portion of the body. It is triangular in shape, with one of the corners anterior, and is about one-third of the entire width. The other two patches are on the ventral surface, one on either side near the posterior end of the cephalothorax, and are much smaller than the dorsal one and of no definite shape.

As the nauplii develop these patches enlarge and deepen in color, and as a result the external egg-cases, which up to the present stage have been a uniform pale yellow, gradually show the color of the patches through their walls and become more and more brown, until this hue has become uniform.

*Internal structure of the nauplius.*—Fig. 12 is a longitudinal horizontal section through the body of the nauplius. It includes quite a portion of the first antennæ (*a'*), simply the bases of the second pair (*a''*), the rudiments of the upper lip (*lb*), and mandibles (*md*), the side walls of the body (*w*), and a section through the abdomen (*ab*). Between the bases of the antennæ may be seen the large frontal gland in process of formation (*fg*). The entire center of the body is filled with yoke globules, among which are scattered the large spherical vacuoles. As can be seen the digestive system has not begun to develop at this early stage, but first appears a little later. It was impossible to distinguish any eye in the living nauplius and this series of sections reveals no trace of it in the internal anatomy.

There is an extremely rudimentary eye formed in the metanauplius, as we shall see later, but it quickly disappears without leaving any traces behind.

*Transformation of the nauplius into the metanauplius.*—This first portion of the stage which by courtesy we may call the nauplius is gradually transformed into a real metanauplius. The transformation consists in a jointing of the first two pairs of appendages, the appearance of the upper and under lips and all the mouth-parts, the formation of two thoracic segments in front of the abdomen, and the development on each of a pair of swimming legs. At first there is only one of these free thorax segments, while the abdomen is broad and spatulate. But another segment is soon formed, and the abdomen is relatively narrowed. On each of these segments is developed a pair of rudimentary legs, whose long plumose setæ are at first turned forward and inward along the sides of the body. As development progresses the position of the setæ changes, and they turn gradually backward, until in the completed metanauplius they point backward and inward (fig. 15).

*Body form of the metanauplius.*—On freeing a metanauplius from its surrounding envelopes in the same way as was done with the nauplius, it presents the general appearance seen in fig. 16. The body is very thick and stout; in fact, nearly spherical, as it was inside the egg, and there have been radical changes in the appendages. The first antennæ (*a'*) are now divided into three segments, of which the terminal is much longer than the two basal, and are still each tipped with two plumose setæ. The second antennæ (*a''*) have a five-jointed exopod, each joint ending in a plumose seta, and a two-jointed endopod, which retains the two terminal setæ, but carries at their base (fig. 17) a well-developed claw inside of the skin. The upper lip is elliptical in shape, wider than long, and evenly rounded, with a small protuberance on either side near the posterior edge.

Just behind the upper lip and a little outside of it lies on either side a short, wide, and bluntly rounded protuberance, one of the paragnaths, which eventually unites with its fellow on the opposite side to form the lower lip. Between the base of the upper lip and the paragnath projects the mandible (*md*), which is short, uniramose, and tipped with a single seta.

The first maxilla (*mx'.*) is biramose at the tip, the outer ramus much shorter than the inner and each armed with short spines.

The second maxillæ (*mx''.*) are stout uniramose appendages, longer and larger than either pair of antennæ, indistinctly three-jointed and terminated by a weak and slender claw.

The basal joint lies outside of the first maxilla and this appendage does not properly appear in the section shown in fig. 18. It has been introduced, however, from the second preceding section in its proper place with reference to the other appendages. The maxillipeds (*mxp.*) lie inside of and distinctly posterior to the second maxillæ; they also are uniramose and indistinctly three-jointed, and terminate in a stout claw with a small protuberance at its base. The two pairs of swimming legs are very rudimentary, each leg consisting of a basal joint and two one-jointed rami, armed with long plumose setæ. The anal laminæ are as large as the rami of the legs, and are also armed with long plumose setæ.

*Internal structure of the Metanauplius.*—On examining the section shown in fig. 18 it can be seen that there has been considerable internal development. The fine-grained protoplasmic masses at the anterior and posterior ends of the body have increased greatly in size, and the muscles which are to move the appendages are fairly well differentiated.

This is especially true of the powerful muscles which are to operate the swimming legs and which appear on the dorsal side of the body near the posterior end of the cephalothorax (*m*).

The mesenteron (*ms.*) epithelium has also begun to be formed around the ventral surface of the stomach, but is incomplete on the dorsal surface. From this ventrally formed stomach layer a long process extends backward which is to become the intestine, while another shorter one is given off toward the mouth. The space within the epithelium is still filled with tightly packed yolk globules, interspersed with large vacuoles, and contains enough nourishment to supply the larva for some time to come. This being just previous to hatching so that the internal structure is approximately the same as during the following stage, we can readily see from the condition of the digestive tract that the larva will not be able to take any nourishment during the first copepodid stage. Such a condition must be kept clearly in mind since it will profoundly influence our interpretation of the free-swimming period.

The extremely rudimentary eye (*e*) can now be distinguished inside the coils of the attachment filament. It is made up of three ovate ocelli, two dorso-lateral and one inferomedian, which are entirely separated from one another and devoid of pigment. The structure of each ocellus has also degenerated until all that remains is a more or less granular mass, staining deeply in hæmatoxylin and containing near its anterior end three lighter spots. No trace of lenses can be found in any of the sections and the entire structure disappears during the next stage.

*Attachment filament.*—By far the most interesting structure in the body of this fused larva remains to be described. This is the attachment filament, already mentioned, which can be seen close to the dorsal surface of the body beneath the patch of brown pigment (fig. 10). It is of interest not only by reason of its intrinsic anatomy, which is very peculiar, but also on account of the function which it subsequently performs. It is an organ which in other parasitic copepods appears at a much later stage, but has here been shifted back to the very beginning of larval development.

It also differs radically from all other attachment devices and is admirably suited to the complicated function it has to perform. In the Caligidæ the filament is manufactured during the process of attachment out of the viscid secretion of a gland, which hardens when it strikes the water. Previous to attachment the larva swims about freely.

In the present species the filament is of solid tissue and is formed slowly inside the body of the larva during the long period it passes within the egg. It is fully developed before the larva is hatched, which is a strong indication that the free-swimming period is to be very short, and we find this fully realized. Furthermore in the Caligidæ the larva is only attached through two or three moults and then becomes free-swimming again. In our *Achtheres* larva the first attachment is maintained by the female throughout life and by the male up to the time of complete sexual development.

The organ of attachment consists of a long filament coiled up like a rope, the two ends extending forward to the frontal margin and considerably enlarged. The ends and the straight portion in front of the coil are surrounded by a large mass of glandular tissue which evidently secretes the filament (fig. 13). The development of this organ is as follows: There appears first very early in the nauplius stage an oval mass of glandular cells (*fg.*, fig. 12) on the midline, close to the frontal margin. This is the frontal gland and corresponds with the one which appears in the chalimus larva of the Caligidæ.

Similarly this gland shows a division into right and left halves along the center. There arises out of it, on the midline between the two halves, a homogeneous body which is at first shaped exactly like a mushroom (*ms.*, fig. 34). The umbrella portion at the anterior end is about four times the diameter of the stalk, and lies with its rounded top in contact with the inside surface of the nauplius epithelium at the frontal margin. The stalk extends backward along the median line and then curves over dorsally and terminates in a small peg (*p*), which lies above and just behind the umbrella enlargement.

This peg is the point of attachment between the filament and the gland, through which the secretion of the latter is conducted into the former (fig. 13). Every part of the organ, umbrella, stalk, and peg, is hollow and is filled with the secretion from the gland. This secretion is a stiff, homogeneous, adhesive substance, which hardens into the filament and is indistinguishable from it in color and transparency.

In his description of the larva of *Achtheres percarum*, Claus says that he regards this organ as the duct of the gland which furnishes the secretion. The present author is obliged to take issue with such an interpretation for the following reasons: 1. The distal end of the filament is completed at the very first, and subsequent growth takes place at the proximal end, the new portion being pushed out against that already formed and gradually coiled up to economize space. The growing point of the filament is thus its point of attachment to the gland. Ducts are not formed in this manner; they begin at the gland and grow away from it, the growing point being at the distal end. 2. The walls of the filament are absolutely homogeneous and transparent, even when first formed, and show none of the structure ordinarily found in ducts. 3. The filament is manufactured directly from the secretion itself; gland ducts are never the product of the secretion of the glands. 4. It is the filament which becomes the attachment organ, and not the secretion which it contains, as will be explained later. A gland duct that was afterwards torn away from the gland and made to serve as an attachment organ would be an anatomical novelty.

When this filament is first formed the stalk is comparatively short and straight (*a*, fig. 14). But as the secretion accumulates the stalk increases in length. Since the two ends are fastened at the very beginning the only way for an increase in length to take place is by a twisting and subsequent coiling of the stalk, and this is what occurs.

At first the stalk twists back and forth (*b*, fig. 14), or into a corkscrew shape, and then, extending back into the tissue behind the gland, it begins to coil. Eventually when fully developed it consists of three large coils, which surround the rudimentary eye, and the two straight portions, which extend to the frontal margin (fig. 34). The total length is considerably more than the entire length of the larva; as a duct such an increase in length would be difficult to explain; as an attachment filament it explains itself.

As already stated, the increase in length takes place at the proximal end where the filament is brought in contact with the glandular substance by means of the peg. This peg is slightly different in color and structure from the rest of the filament, and stains differently. At the point where it actually joins the gland it spreads out into a sort of funnel, whose walls are extremely thin and delicate (fig. 13). The walls evidently form a viscid film on the outside of the secreted substance, plastic enough at the proximal end to be pushed along gradually by the accumulating secretion, but rapidly growing thicker and firmer distally. The new film constantly forming at the point of union thus becomes continuous with that which has preceded it, and in this manner the entire organ is formed.

Nordmann mistook the ends of the filament for the eyes of the larva, while he supposed the coiled portion to be connected with the mouth-parts. But he makes the following pertinent statement:

> The length of the filament (Röhre) is in direct relation with the development and size of the embryo. The longer it is, so much the farther has the development of the animal progressed; the shorter it is, so much the younger is the embryo.

This is exactly true of the present species, and the fact that this filament is completely developed before the larva escapes from the egg indicates that it is to be used *at once.*

*Hatching.*—The larva is now ready for hatching. This process in the present species is similar to that which has been well described by Claus for *Achtheres percarum.* The thick wall of the egg sack is so constituted that it becomes weak and brittle through the action of the water at just the time when the larvæ have completed their development and are trying their muscles. Through both of these influences, as well as by osmosis, the sack bursts open at one or more places and the larvæ pass out into the water. They are still surrounded by the various membranes that have been formed during their development, but these are quickly and easily gotten rid of.

As soon as the water strikes the egg membrane, which it will be remembered is formed of the same cement substance as the external sack, it swells a little and bursts from the osmotic pressure. The inner membranes are very thin and delicate, and are easily ruptured by the struggles of the larva, which are increased when subjected to the change of environment. In this way it comes about that the molting of the nauplius cuticle is simultaneous with the escape from the egg, or follows it after an interval of only a few moments.

With reference to the actual period of hatching, Nordmann stated that the larvæ of *Achtheres percarum* hatch directly into the first copepodid stage. Kollar's observations on *Basanistes huchonis* were interrupted, and he was not certain whether there was a brief metanauplius stage after hatching or not. Beneden makes no statement on this point, but he gives us a figure of a metanauplius larva free from enveloping cuticles. Vejdovský states with reference to *Tracheliastes polycolpus* that the metanauplius stage is passed wholly inside the egg, the larva hatching in the first copepodid stage. Kellicott, in describing the larva of *Achtheres corpulentus,* states that he removed some of the brood from the eggs to make sure, and "found them in the form of the swimming ones," that is, in the first copepodid stage. Claus stands alone in his statement that the larvæ of *Achtheres percarum* hatch in the metanauplius stage and molt into the first copepodid stage "after a few hours."

From a careful examination and comparison of a large number of egg strings of *Achtheres ambloplitis* during the period of hatching, the present author has come to the following conclusions:

1. The great majority of the larvæ hatch directly into the first copepodid stage, and it is the evident expectation that all should do so. Under natural conditions it is probable that at least 95 per cent hatch in this way.
2. The actual emergence of the larvæ depends on the texture of the external egg sacks. If they soften and crack rapidly the eggs may be let out into the water early enough for the larvæ to escape as metanauplii. This is more likely to occur if the females have been handled and kept in aquaria than under normal conditions.
3. The few metanauplii thus obtained are all ready for the molt into the copepodid stage, and this occurs in from ten or fifteen minutes to an hour. Such metanauplii are too stocky, their locomotor organs are too weak, and it is too near the molting time for them to move around much. All they can do is to lie inertly on the bottom of the aquarium until the molt into the copepodid stage. Under natural conditions such premature hatching with its attendant helplessness might easily prove fatal to the larva.
4. Ordinarily the egg sack does not break open until the movements of the larvæ become quite energetic. The transformation into the first copepodid form is effected inside the egg membranes and the larva comes forth a vigorous and active swimmer.

In this species therefore the early larval stages up to the molt into the first copepodid stage are so thoroughly fused as to become one single period. There are no definite points of which it can be said, these mark the completion of one stage and the beginning of the next. It is rather one continuous development, and the peculiarities which usually characterize the different stages overlap one another, so that those belonging to several may be present at the same time. The only point of reckoning is the formation of the nauplius cuticle, and even this occasions no break in the continuity. Development goes on inside of it through the nauplius and metanauplius stages just as if it were not present.

## FIRST COPEPODID OR FREE-SWIMMING STAGE.

### BODY FORM.

As the larva emerges in this stage from the egg it appears in a form which, by the segmentation of the body and by the structure of the appendages, corresponds with the first cyclops stage of free-swimmers (fig. 23). The body is much elongated and flattened dorso-ventrally; the cephalothorax is elliptical, and nearly twice as long as wide, the proportion being as that of 9 to 17. It is enlarged at the anterior corners above the bases of the second antennæ, and furnished with a distinct notch on either side and a dorsal groove between the head and first thorax segment. It is slightly arched dorsally, but flat on the ventral surface, and carries at its posterior end a wide obcordate process, which projects over the base of the second thorax segment.

The shorter and smaller posterior portion of the body is made up of four segments, sharply separated from one another, the last one carrying the broad anal setæ.

The first of these segments, the second thoracic, is the largest and the third one is the smallest. The first one is half the width of the cephalothorax and has a broad, semicircular, posterior process. The remaining segments are all the same width, which is half that of the first segment.

On each side of the second one at the center is a small papilla, armed with a single long spine, the rudiments of a third pair of swimming legs. The last segment, which is a fusion of the posterior thoracic and abdominal segments, is subquadrilateral in outline, with nearly straight sides.

The anal laminæ are large and broad, and each carries three long and three short setæ. The total length of this larva is 0.48 mm. Length of cephalothorax, 0.32 mm. Width of same, 0.165 mm. Length of free thorax, 0.12 mm. Width of first free segment, 0.08 mm.

These larvæ are transparent and colorless except for four patches of dark brown pigment, one dorsal, the other three ventral. The dorsal patch corresponds to that already described in the nauplius, but has enlarged considerably.

The posterior ventral patch includes the two ventral ones of the nauplius, which are now darker in color and have enlarged until they are fused across the midline (fig. 22). In addition, there is a small anterior patch on either side just behind the base of the maxilliped.

### APPENDAGES.

The first antennæ are attached on the dorsal surface, are cylindrical, uniramose, and four-jointed. The first and third joints are each armed with a single seta, the second with two, while the terminal joint ends in a cluster of five.

The second antennæ are attached on the ventral surface, beneath the bases of the first pair (fig. 22). They are biramose, the exopod one-jointed with a blunt and smoothly rounded tip armed with a single spine, the endopod two-jointed and ending in a strong curved claw. Between the bases of the second antennæ on the ventral surface is the conical mouth-tube. This ordinarily projects downward and forward, and reaches a little beyond the anterior margin of the cephalothorax. It is as wide at the base as it is long, and is bluntly rounded at the tip, with a fringe of short hairs around the terminal opening. It is made up of a rather flat and narrow upper lip, and a fluted and grooved lower lip, whose edges overlap those of the upper lip, and whose tip is arched into a cylinder to form the suction opening. This lower lip is made up of two halves which arise from separate protuberances, the paragnaths, on either side of the midline and behind the edges of the upper lip (fig. 16), and are not yet fully fused at the tip. Outside the base of the mouth-tube lie the mandibles and first maxillæ. The former are uniramose and one-jointed and are tipped with two short and stout spines. The latter are also uniramose but two-jointed, the terminal joint ending in a single spine. They also carry on their inner margin a short cylindrical palp tipped with two small setæ.

The second maxillæ are some distance behind the first pair and close to the margin of the carapace. Each is stout and two-jointed, the terminal joint ending in a long and slender claw bent into a half circle. The maxillipeds are close behind the second maxillæ, but are considerably nearer the midline. Each is three-jointed, the terminal joint tipped with a stout claw, nearly straight except at the very end. These maxillipeds are the chief organs of prehension and are considerably longer than the second maxillæ.

The two pairs of swimming legs are biramose, the basal joints broad and laminate and furnished with powerful muscles, the rami small, one-jointed, and about as wide as long. The exopods of each pair terminate in four stout and long plumose setæ, while the endopods have six.

### INTERNAL ANATOMY.

Internally there has been but little change from the preceding stage. The frontal filament is now completely developed, and may be seen close to the dorsal surface, with the mushroom end flattened against the inside of the frontal margin. The opposite end is also enlarged and fastened into the tissue just back of the mushroom. The peg has disappeared, its function having been performed. The eye of the larva is invisible externally, but it is probably present inside the coils of the attachment filament in the same degenerate state as at the close of the preceding stage.

The epithelium of the mesenteron which began to be formed in the metanauplius has now developed further and entirely surrounds the mass of yolk globules. But the stomedeum and proctodeum are not yet completed, and the larva is therefore incapable of taking nourishment. Claus has described this first copepodid stage in *Achtheres percarum* as possessing a completed digestive system, capable of functioning. But he stands alone in such a statement. Nordmann, Kollar, Vejdovský, and Kellicott all represent the larval digestive apparatus at this stage as only partially developed, and thus agree with what is found in the present species. All the available accessory testimony also witnesses to the same fact, that the larva at this time is incapable of procuring or digesting food.

The digestive system did not begin to develop until the latter part of the preceding stage and did not advance very rapidly. At the close of the stage when the larva was ready to molt into the copepodid form, the mesenteron was not yet completed while the stomodeum and proctodeum were scarcely begun. It would be impossible for all three to be finished and ready to function in the short time consumed in molting. Again, the mouth-tube is not yet completed. The halves of the under lip, which are to form the sucking tip of the organ, have not yet grown together and could not, therefore, perform their destined function. Moreover, the mandibles are still on the outside of the proboscis and in a very rudimentary state, entirely unfitted for piercing. This combined testimony practically proves that the larva is nourished by the abundance of yolk still left in its stomach until after it has attached itself to a host.

### SEARCH FOR A HOST.

In its search for a host the larva swims about actively, the long rowing setæ furnishing powerful locomotor organs. The motion is not at all jerky like that of the free-swimmers, but is smooth and rapid like that of an *Argulus* or *Caligus*. In an aquarium this energy is kept up for about twenty-four hours and then relaxes, the larva by the second day becoming so wearied that its feeble efforts scarcely move it at all. In fact, it has been the common experience of all investigators that such larvæ usually die within thirty-six hours unless thay find a host. This fact, combined with the complete development of the attachment filament and the rudimentary and incomplete condition of the mouth-parts, indicate strongly that this is a short transition period, just long enough to bring larva and host together.

The present species infests the Centrarchidæ, and fishermen are well aware that these fish are in the habit of catching their food at or near the surface of the water. And this is the place where the *Achtheres* larva awaits its victim. In seizing its prey, as well as in the act of ordinary breathing, a fish takes in water through its mouth and expels it through the gills. The *Achtheres* larva would be easily swept in at such a time by the incurrent water, and when brought in contact with the gills by the outcurrent its powerful grasping organs would enable it to secure a firm hold on the gill filaments or arches.

It is worthy of notice in this connection that those of the Centrarchidæ which feed most persistently at the surface are the very ones most infested by these parasites. The red-eye, *Ambloplites rupestris*, is the common host of the present species.

### FIXATION TO THE HOST.

Of course, it is practically impossible to actually witness the fastening of the larva. But from what has been observed in the chalimus larva of the Caligidæ,[a] we can infer what occurs here. The outer end of the attachment filament is enlarged into the mushroom form already described and is filled with adhesive fluid. It lies just inside the frontal margin, covered only by the very thin outer cuticle (fig. 23). Doubtless, the larva rubs its frontal margin against the skin of the gill arch of its host and in this way burrows through the slime and outer integument to the solid tissue underneath, holding on meanwhile with its powerful maxillipeds. At the same time the thin covering of the frontal margin of the parasite is broken through and the end of the filament is brought in contact with the gill arch, to which it adheres firmly. By moving away from the point of attachment, the coiled filament is drawn out of the body of the larva. As it comes forth the larva grasps it between the claws at the tips of the second maxillæ. These claws are bent into a half circle whose diameter is one-half larger than that of the filament. The latter can thus slip through the claws easily, but by shutting past each other, as they naturally do, the claws can still retain a firm hold. When the enlarged posterior end of the filament is reached, it is held securely between the claws and is removed entirely from the frontal margin. The attachment filament thus becomes fastened to the ends of the second maxillæ, where it afterwards remains throughout life.

That this transference of the filament from the frontal margin to the tips of the second maxillæ takes place at the time of fixation is manifest from several considerations.

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[a] Proc. U. S. Nat. Mus., vol. 28, p. 546.

First, no larvæ have ever been found fastened by their frontal margins. Nordmann was fortunate enough to discover a crowd of the copepodid larvæ clinging to the roof of the mouth of a perch. On removing them and placing them in water, it was found that some swam around vigorously in the water while others crawled about by means of their maxillipeds.[a] The former had probably just found the fish, while the latter were ready to attach themselves.

Considering that the locomotor organs of these latter, in bringing them to the fish, have entirely served their function and are to degenerate and disappear at once, it seems probable that they cease activity as soon as the larvæ are once fastened to their host, and become practically useless before the moult occurs. At all events, none of these larvæ were fastened to the fish by their frontal margins, for this would scarcely have escaped Nordmann's observation.

The present author has secured every copepodid stage from the adult down to one which was shorter than the free-swimming form just described, and into which the latter molts. But this larva was fastened by the tips of the second maxillæ and not by the frontal margin. Again the second maxillæ, when first found with the filament attached to them, are too short to reach the frontal margin. We can easily understand how at the time of attachment, while the larva is still clinging with its maxillipeds, it could pull its body forward, after the end of the filament was attached to the gill arch, far enough to allow the maxillæ to grasp it. But it is not easy to see how these maxillæ could get hold of the filament after the larva had once cast itself loose from the fish and was hanging by its frontal margin. Since the second maxillæ are to serve as attachment organs throughout life, there is every reason why the transference should be made at once and no excuse to offer for the postponement of it.

## SECOND COPEPODID STAGE.

### BODY FORM.

Claus predicted for this stage a larva in which the number of body segments was not increased, the mandibles were inclosed in the mouth-tube, and the setæ of the swimming legs had degenerated. He was unable to find such a theoretical larva, but it can now be presented, and the closeness with which it conforms to his prediction proves the accuracy of his interpretation.

This larva is actually shorter than the free-swimming form, owing to a refusion of the segments there separated (fig. 28).

The body has been much thickened dorso-ventrally, so that it is no longer flat but cylindrical, while the thorax and abdomen have been relatively enlarged, and all the joints have stiffened so as to be practically immovable.

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[a] Mikrographische Beiträge, zweites heft, p. 84.

The general shape of the female is that of a spindle, the thickest portion being through the bases of the maxillæ and maxillipeds, the anterior cone much shorter than the posterior.

The first thoracic segment has separated from the head as distinctly as any of the others and is as wide as the cephalon; the others diminish in regular order backward.

The five segments in the body of this larva are thus due to a further separation of the first segment from the head and not to the formation of a new segment. There are really just as many as there were in the first copepodid stage.

On the dorsal surface the dividing groove between the second and third segments is deeper than the others; on the sides and ventral surface they are all equal.

The anal laminæ are much reduced in size and consist of short and stout papillæ, projecting from each posterior corner of the abdomen and tipped with four tiny spines.

### APPENDAGES.

The first antennæ are three-jointed, the two terminal joints having fused, and are only sparingly armed with short setæ.

The second pair (fig. 27) are very similar to those of the free-swimming larva, but project farther. They are made up of a long and stout basal joint and two rami; the exopod is indistinctly two-jointed and ends in a stout curved claw, with an accessory spine at its base; the endopod is one-jointed and tipped with two minute spines (fig. 30).

The mouth-tube (fig. 26) is more fully developed, the upper lip having increased in size and the halves of the lower lip being thoroughly fused. The mandibles have entirely changed and are now piercing organs, inclosed within the mouth-tube, but easily slipped outside through the slit in the side of the tube between the edges of the two lips (fig. 31). They are attached at the base of this slit; each is enlarged at the proximal end, tapers into a narrow shank, which is curved over ventrally, and ends in another enlargement, with six or eight curved teeth along its ventral edge. They reach about two-thirds of the distance from the point of insertion to the tip of the mouth-tube.

The first maxillæ (fig. 31) are now attached directly to the sides of the lower lip at a point a little above (distal to) the insertion of the mandibles. Each is uniramose, as long as the mandible, and tipped with two spines of equal length, with a very much shorter one at their base.

The second maxillæ have entirely changed; they still remain short but have lost all trace of segmentation and are much swollen laterally, so that they are three-fifths as wide as long (fig. 20). The end of each is reentrant or bowl-shaped, and the claw is attached in the bottom of the bowl. It is very short and stout, with an enlarged, bluntly rounded or conical tip; the base of the cone forms a flange or barb around the claw and is reentrant at one point on the ventral side (fig. 21). This claw is entirely without muscles and therefore can not be moved, except as the whole maxilla moves.

The two claws can be distinctly seen imbedded in the enlarged proximal end of the attachment filament, and can be easily pried out of the latter (fig. 19). The filament also is a hollow tube whose structure corresponds exactly with that seen in the frontal region of the larva in the preceding stage. And if we follow along to the distal end of the filament, where it is attached (in this instance) to one of the spines on the gill arch, we find there the mushroom-shaped enlargement which was so conspicuous in the free-swimming larva (figs. 27 and 38). There can be no doubt of the identity of the two structures. It often happens that the claws and the filament for some little distance near them are covered with a mass of the adhesive substance arranged in ridges or transverse wrinkles, as though some of the contents of the tube had escaped when it was transferred from the frontal margin (fig. 24). This is exactly what would be expected and strengthens the proof of such transference.

The maxillipeds retain their segmentation and have practically the same structure as before. The only change has been that they have migrated forward a little between the bases of the second maxillæ, so that now the two pairs of appendages are about on a level.

The swimming legs have greatly degenerated; they have diminished in size until oftentimes it is difficult to find them and they have lost their plumose setæ. They are now made up of a tiny basal joint and two still more minute rami, each of which is tipped with two short spines (fig. 28). They disappear entirely at the next molt. The spines which represented the third pair of legs on the sides of the third thorax segment in the free-swimming stage have entirely disappeared.

Total length of this second copepodid larva, 0.42 mm. Length of cephalothorax, 0.30 mm. Width of same, 0.20 mm. Width of second thorax segment, 0.10 mm.

### SEX DISTINCTION.

The larvæ show enough differences in this second copepodid stage to enable us to distinguish the sexes.

The male (fig. 25) is shorter and more stocky than the female; in dorsal view the body is distinctly separated into two regions, a cephalon subquadrilateral in outline and nearly half the entire length, and the body proper, cylindrical in form, narrower than the cephalon, and tapering backwards. Between these two regions is a strong constriction, extending entirely around the body and making a sort of waist. This causes the posterior portion, the body proper, to appear very much like the abdomen in the Hymenoptera. The pigment, which in the female (fig. 28) is confined to a single spot on the dorsal surface of the carapace at the center, is in the male distributed as a narrow dendritic line along either margin of the carapace in addition to the central spot.

The second maxillæ and maxillipeds are larger and stouter in the male, longer and more slender in the female. Especially is this true of the second maxillæ, which now are the attachment organs. In the male they are little if any longer than the maxillipeds, and they *do not increase in length* as development progresses. On the contrary they remain about the same length and retain their terminal claws, together with all the musculature connected with them.

In the female, although the second maxillæ may not be very much longer than the maxillipeds when they first take over the attachment filament, they rapidly increase in length until they become fully twice or three times as long. At the same time the musculature is withdrawn from connection with the terminal claws, and finally the claws themselves disappear.

Of course in subsequent development size enters as a distinctive factor and quickly becomes predominant over the differences just mentioned. The male is to be a pigmy when sexually mature, and hence increases in size very slowly, and never gets to be over a millimeter in length. The female, on the other hand, grows normally and is 4 or 5 millimeters long when fully developed.

The later the stage of development, therefore, the greater will be the size difference between the two sexes, and a point is soon reached where this factor alone will enable one to recognize the sex.

There is very little difference in the appendages between the two sexes. The first antennæ of the male are stouter and longer than in the female, and have only the terminal tuft of three short setæ. The second pair are relatively shorter, so that the tips of the two pairs in the male are on a level. There is a rather larger knob at the base of the terminal claw of the exopod, and the endopod has three tiny spines on its tip. The mandible reaches more closely to the tip of the mouth-tube, but easily slips outside of the tube as in the female. The first maxillæ are shorter and stouter, and the two terminal setæ are more conspicuously jointed at their base. These maxillæ hardly reach beyond the base of the mandible, while in the female they reach its tip (compare figs. 26 and 31).

### DIGESTIVE SYSTEM.

Interest of course is centered chiefly on the internal anatomy of the two sexes at this period. On examining a median longitudinal section (fig. 32) of the male larva shown in figure 24, and of a slightly larger female larva (fig. 33), we find that the digestive canal traverses the entire length of the body, its two openings being virtually terminal.

The mouth-tube is turned forward and its tip projects in front of the frontal margin, making it the most anterior portion of the body. Through its center runs the slender thread-like œsophagus (*oe.*) which extends back opposite the base of the second maxillæ without any turn or sharp bend. There it passes abruptly into the enlarged stomach, entering the center of the anterior end in the male and the anterior ventral corner in the female. At the point of junction a thick sphincter muscle (*sm.*) is formed, which projects strongly into the stomach (*s*). The latter is completely fused with the intestine so that it is impossible to distinguish any point of separation. Both possess thick walls made up of an outer serous membrane, set with nuclei and serving as the point of attachment for the numerous fibers and muscles which suspend the tube within the body cavity, a median muscular layer which produces the peristaltic movements, and an inner glandular layer composed of large cells, whose darkly pigmented contents are gathered around a much lighter nucleus. Some of these cells project into the stomach cavity much farther than others, and their inner free ends are filled with a digestive secretion, as evidenced by differential staining (*gc.*). In the male the intestine stops abruptly at the anterior margin of the last (abdominal) body segment. The entire abdomen beyond it is filled with a plug of loose cells and muscle fibers, through the center of which may be seen the proctodeum (*pc.*) in process of formation. But it is not yet finished and the anus has not broken through, the posterior walls of the abdomen being entire.

In the female larva, however, the posterior portion of the digestive tract is fully formed; otherwise there is no difference in this region between the sexes. At this early stage the body cavity around the digestive tract is open save for the grouping of cells in various places to form the beginnings of other organs. Through the wide spaces thus left the blood can circulate freely.

### FRONTAL GLAND.

In the anterior portion of the head, dorsal to the œsophagus, is the large secretory gland which formed the attachment filament in previous stages (*fg.*). The filament itself is gone, and the peg or attachment end has been torn out, leaving a gaping hole filled with a plug (*p*) made of the secretion of the gland. This furnishes the final proof of the transference of the filament from the frontal margin to the tips of the second maxillæ. Claus thought he discovered the remains of the peg in the head of the larva which he has figured, but he was judging, as he admits, entirely from external appearances. The nature of this plug is clearly proved by the way in which it stains. It is not the peg, for that remains uncolored in both hæmatoxylin and eosin; it is not part of the gland itself, for that takes a deep-blue stain; it is the secretion of the gland, which stains deeply with eosin. The very rudimentary eye, which was found in the nauplius-metanauplius stage within the coils of the attachment filament, has entirely disappeared. It goes without saying that there can be no remnant of the lenses left, for there were no lenses to begin with in this species. Moreover, there was no pigment in the larval eye, and hence the irregular pigment spots found on the dorsal surface of this larva, above the brain, can not be explained as "taking their origin in the pigment of the larval eye," which is the explanation given by Claus for *Achtheres percarum.*

### EXCRETORY GLANDS.

In addition to these frontal glands, there are several excretory glands in the head, some of which are more or less connected with one another. The largest starts at three different centers on the level of the bases of the maxillipeds. One center is median and dorsal, between the wall of the anterior end of the stomach and the dorsal body wall (*mpg.*, fig. 32). This may be designated as the median center of the maxillipedal gland. It begins as two or three ellipsoidal balls or accretions of very small cells, gathered just beneath the ectoderm in this region of the back. These are supported by a fine meshwork of connective tissue from the dorsal ectoderm, the outside layer of the stomach wall, and the adjacent dorso-ventral muscles, which operate the second maxillæ and the maxillipeds (fig. 37).

At first these glandular masses are small and, especially in the female, are often spherical; but as they increase in size they accommodate themselves in both sexes to the shape of the cavity in which they are formed. Elongating antero-posteriorly, they become ellipsoidal or cylindrical and often taper into cones (*mc.*, fig. 37). Owing to the position of the ovaries or testes close behind them on the median line, these masses move around to the sides of the stomach as they increase. Here they grow backward until they overlap considerably the anterior end of the sex organs. Sometimes this lateral movement eventually draws the masses away from the midline, leaving that space free for the maturing ovaries and testes.

As soon as this median portion of the gland appears, a similar mass may be found on either side near the bases of the maxillipeds (*lc.*, fig. 32). These lateral maxillipedal centers increase similarly to the median one, and finally fuse with it around the sides of the stomach. A spirally convoluted duct (*d*, fig. 37) is then formed, leading from the fused lateral mass on either side to the base of the maxilliped, where it opens to the surface. The walls of this duct are composed of a thick homogeneous layer, set with nuclei, and taking both the red and the blue stains.

Another pair of glands, situated in the bases of the second maxillæ, one on either side, may be called the maxillary glands (*mxg.*, fig. 37). Each is a single well-rounded ellipsoidal mass, nearly filling the cavity of the maxilla and giving off from its inner surface at the center of the ellipse a straight duct, which leads toward the distal end of the basal joint, where it opens to the surface on the inner side.

As development proceeds the maxillipeds migrate forward, taking the distal end of the convoluted duct with them, while the second maxillæ in the male point diagonally backward outside of the maxillipeds. It thus comes about in the adult that the bulk of the maxillipedal glands is behind the appendage at whose base their duct opens, but in front of the maxillary glands. Such a relative position would be difficult to explain if we did not have the developmental stages in which to follow the various changes.

### NERVOUS SYSTEM.

The nervous system is made up of a very large infra-œsophageal ganglionic mass and an equally small supra-œsophageal portion. The latter can hardly be distinguished in the female from the walls of the gullet (*sog.*, fig. 33). At its anterior end two nerves are given off on either side, one beneath the other. The upper and smaller one (*a'*) goes to the first antenna, but gives off a branch near the base of the antenna which supplies the frontal margin. The lower and larger one (*a''*) goes to the second antenna, but sends a branch to the upper lip. At its posterior end this supra-œsophageal ganglion gives off a large nerve (*n*, fig. 32) on either side, which runs along the anterior wall of the stomach and supplies the dorsal portion of the head, the frontal gland, and the maxillipedal excretory gland.

From the anterior end of the large infra-œsophageal ganglion a good-sized nerve trunk (*lb.*) runs forward to the base of the lower lip, where it divides and sends a branch to the mandible (*md.*).

A slender nerve, given off from the ventral surface of the ganglion, goes to the first maxilla (*mx.*). Another larger one (*mx'.*) just behind it supplies the second maxilla, while from the postero-ventral corner a still larger nerve runs to the maxilliped (*mxp.*). Near the postero-dorsal corner a delicate nerve thread runs down diagonally to the lateral maxillipedal and maxillary glands (*mpg'.*). From the corner itself is given off the slender nerve which extends backward along the ventral wall of the stomach and close to its fellow from the other side. These ventral nerve trunks (*vc.*) are no larger than those which supply the maxillipeds, and although they give off branches to the stomach and body walls, to the various muscles, and to the reproductive organs, they show no ganglionic swellings, and no ganglion cells.

The nervous system, therefore, is practically all concentrated in the infra-œsophageal ganglion, since the part above the gullet is hardly swollen more than enough to serve as the origin of the nerves that arise from it. In this respect the Lernæopodidæ present a strong contrast to the Ergasilidæ and Caligidæ, as also to the free-swimming forms.

The infra-œsophageal ganglion is made up of a peripheral layer of nerve cells and a central mass of fibers. The cells are evenly distributed over the outer portion of the ganglion and are not bunched at the origin of the nerves.

There are a very few cells in the commissures at the sides of the œsophagus and in the supra-œsophageal ganglion, but the great mass of both these portions is fibrous, and nothing but fibers can be found in any of the nerve trunks or branches.

### REPRODUCTIVE ORGANS.

The beginnings of the reproductive organs are shown in both sexes at this second copepodid stage. The ovaries and testes are paired and lie in what may be termed the small of the back, between the stomach and the dorsal body wall and just behind the median maxillipedal gland (*t*, fig. 32 and *o*, fig. 33). The ovaries begin as small spherical masses of cells, not very definitely arranged, but gathered together on either side of the midline (*o*, fig. 36). From the posterior end of each is given off a slender oviduct which runs around the stomach wall to the ventral surface and then backward to the opening in the side of the genital segment. At intervals along the duct are gathered rounded masses of cells which later develop into the uterine processes, within which the eggs are matured (*up.*). At the posterior end of the duct is an irregular cellular mass in which may be distinguished the beginnings of the cement gland (*cg.*), its duct being corkscrew-shaped at this stage. Between these two masses in the center of the body is another cellular accretion which later develops into the semen receptacle and its ducts.

As the sex organs mature the posterior portion of the body of the female increases greatly in size, especially laterally, leaving large spaces on either side of the digestive tract. These are filled as fast as they are formed by the increased convolutions of the oviduct and especially by the development of the uterine processes anteriorly and the large cement glands posteriorly. Claus evidently did not see either the ovaries or the testes of *Achtheres percarum*. The bodies which he has designated as such are a long distance back of their true position, as revealed in serial sections. In the living larvæ as well as in the adults the sex organs are so situated as to be invisible from the exterior. But certain portions of the convolutions of the oviducts and vasa deferentia come close to the body wall and are easily distinguished. And these are what Claus has designated as the organs themselves.

The testes begin in the same manner as the ovaries, the separate masses becoming afterwards the lobes of the fully devoloped organs (fig. 35). The deferent duct (*vd.*) at first turns forward to the ventral surface, then swings back to the dorsal surface, runs diagonally to about the center of the lateral surface, where it is greatly swollen, and finally turns diagonally forward again to the opening on the ventral surface of the genital segment. The free portion (*fd.*) of the duct is much larger than the oviduct, chiefly owing to the increased thickness of its walls. In the swollen anterior convolutions of the duct may be distinctly seen the cells which are to be transformed later into spermatozoa.

As development proceeds the deferent duct, like the oviduct, becomes more and more convoluted and swollen, until it finally fills the entire cavity on either side of the digestive tract, and it presses against the latter so much that the rectal portion of the intestine becomes flattened laterally into a mere slit, difficult to distinguish except in transverse sections. In this second copepodid larva the entire posterior segment of the body, which is a fusion of the two posterior thoracic segments and the abdomen, is filled in the male with a mass of cells (*k*, fig. 32) containing fine granules (Claus's "Körnchenhaufen"). These become joined later with the posterior portion of the deferent ducts and supply the cement substance which forms the outer covering of the spermatophores. In this manner practically the entire body cavity behind the cephalon is occupied by the sex organs and the various accessories connected with them.

### CIRCULATORY SYSTEM.

There are no real circulatory organs in this larva or in the adult. The blood moves about freely through the large open spaces in the body cavity, and is driven back and forth by the movements of the digestive tract. Both the stomach and intestine have muscular walls capable of strong peristaltic movements which show much diversity. At one time they sweep forward, at another backward, and again they start at either end and work toward the center, or they may begin at the latter point and move backward and forward simultaneously. These movements carry along with them the blood which lies in contact with the walls of the digestive tract. But the real movement is accomplished in a much more effective manner. To either side of the digestive tract in the third thoracic segment is attached a bundle of muscle fibers which run directly outward and are fastened to the lateral body wall. These contract rhythmically to the right and to the left, pulling the intestine quite a distance away from the midline to the one side and then to the other. The blood, following these lateral movements of the intestine, streams precipitately forward on the side toward which the intestine is pulled, and backward on the opposite side. This motion is then reversed when the intestine is pulled to the opposite side. These movements become stronger with the maturing of the larva, and in the adult they produce a complete circulation.

There are no blood vessels nor any heart. What Claus describes as a pulsating organ ("pulsirende Organ") occupies exactly the position of the median maxillipedal gland, already described. The body is strongly narrowed just posterior to this point and the lumen of the body cavity is tightly filled by the median maxillipedal gland, the lateral glands, the ovaries or testes, and the enlarged anterior end of the stomach. Naturally the blood streams through the narrow interstices between these organs with considerable force and often moves the dorsal portion of the organs with every sway of the digestive tract. When seen through the outer body wall this gives the impression of a pulsating organ, but nothing of the sort can be found in the larva.

### SUBSEQUENT STAGES.

At the next molt both sexes become mature—that is, they are adults, and although they increase afterwards in size, especially the female, there is no further change in bodily structure or the appendages (figs. 38 to 43). One thing, however, is still left to be accounted for, and that is the coming together of the sexes. At present we have them each attached by a filament to the host, whereas we know that in the matured adults the pigmy male clings with his maxillipeds to the body of the female. How is this transfer accomplished by a creature that can not swim?

From this second copepodid stage there is a steady increase in the difference in size between the sexes. The female grows faster than the male, particularly the posterior portion of the body, and the second maxillæ elongate until they are often as long as the entire body. The attachment filament at the same time shortens until all that is left of it is the slender stalk between the attachment disk and the tips of the maxillæ.

In the male the head and body remain approximately the same size; the second maxillæ do not elongate nor does the attachment filament shorten. The claws at the tips of these maxillæ are retained, as are also the muscles connected with them. Indeed, since the filament in this sex does not become fused with the maxillæ, the only way in which the male retains hold of it is by means of the claws driven into its swollen proximal end. The purposes of these differences is now manifest, for they enable us to understand how the union of the sexes takes place.

The females were permanently attached to their host in the first copepodid stage and can not afterward be changed. On the contrary the little motion at first allowed by the long attachment filament is steadily diminished until, by the time the female is sexually mature, it is entirely gone and the tips of the maxillæ are in contact with the gill arch of the host. The female, therefore, is an inactive factor in the union. The male was also fastened by an attachment filament in the first copepodid stage, and his swimming legs degenerated like those of the female. Hence he can not swim in his search for the opposite sex.

When the copepodid larvæ attach themselves to their host several fasten in immediate proximity. The author has repeatedly taken two or three adults from the same spot on a gill arch, each female with an attached male. Sometimes the attachment disks are actually fastened together so that they have to be cut apart, and often the discarded male filaments will be found close beside those of the female.

It is scarcely possible that sexual instinct manifests itself thus early in development, and induces the two sexes to attach themselves side by side. But there may well be a general instinct which leads all the larvæ indiscriminately to keep together during this period. And this is exactly what the finding of several adult females attached to the same spot would indicate.

Having attached themselves as members of such a little group the males, as soon as their sex organs are developed, begin moving around in a circle whose radius is the length of the filament. Somewhere within this space they are pretty sure to find a female; the male then fastens to her body with his maxillipeds and withdraws the claws of the maxillæ from the end of the filament, thus becoming permanently fastened to the female. It is probable that, if the male can not find a female by this method, he is able to withdraw his claws from the filament and crawl about over the gill arch in search of one. The discarded filaments found on the gill arches of the red-eye would indicate at least that the male does not retain this attachment after becoming sexually mature.

## GENERAL SUMMARY.

1. Long filaments of ovarian cells loosen themselves from the epithelium of the ovary and pass down into the uterine processes of the oviduct, where the terminal cells of each filament develop into oöcytes successively, one after another.

2. Each egg is surrounded by a structureless vitelline membrane, within which are yolk globules evenly and universally distributed through a fine cytoplasmic matrix. There are numerous scattered spherical vacuoles of different sizes. The egg nucleus is about the size of the largest vacuoles, is slightly eccentric, nearly spherical, and surrounded by a membrane. It contains a single nucleolus and numerous chromatin granules near the periphery.

3. As the eggs pass out of the oviduct they are each fertilized at the opening of the sperm receptacle, and are covered with a layer of the

secretion of the cement gland, which forms in the external sacks a thick outer shell membrane.

4. The first cleavage nucleus divides and with some of the cytoplasm migrates to the surface at a point opposite the wall of the external sack. The migrated materials are there transformed into blastoderm cells by an accumulation of the cytoplasm around nuclear centers, until four or five cells of the same size have been produced. Then, while the migration continues, a segmentation of these cells also begins and the two processes go on simultaneously until the completion of the blastoderm, when the cells composing it secrete a cuticular membrane, the blastodermic cuticle.

5. After the blastoderm has entirely surrounded the yolk the portion of it which is to become the ventral surface of the embryo thickens by a rapid multiplication of cells, and builds up a series of lobes, which are to constitute the future appendages.

6. The nauplius and metanauplius stages are passed inside the egg, the larva hatching in the first copepodid stage. The first two stages are so completely fused as to be indistinguishable. The nuclear centers of the usual nauplius appendages, of the mouthparts, and of the first two pairs of swimming legs all appear simultaneously. The first and second antennæ develop quickly into normal nauplius appendages. The mandibles never do but, with the other appendages, are arrested for a time, and then develop into the usual metanauplius form. The nauplius eye is so rudimentary that it can be seen only in serial sections; it has neither pigment nor lenses.

7. The most interesting nauplius structure is the attachment filament, begun in the nauplius and completed in the metanauplius stage. It is secreted by a large frontal gland which occupies the whole of the anterior dorsal portion of the cephalon. It consists of a long filament, cylindrical and hollow, straight at first then increasing in length and coiling like a rope, the two ends extending forward to the frontal margin. The distal end is shaped like a mushroom and is the part attached to the host; the proximal end is peg-shaped and is the point of attachment between the filament and the gland.

8. The larva emerges in the first copepodid stage a vigorous swimmer, and at once seeks a host and fastens itself somewhere on the gill arches. During attachment or at least before the next molt, the filament is transferred from the frontal margin to the claws at the tips of the second maxillæ. It remains here during life in the female, being gradually shortened down to the length found in the adult. In the male it remains until the union of the sexes, when the claws are withdrawn from it and the male afterwards clings to the body of the female.

9. The attachment button of the adult, therefore, is the mushroom enlargement at the distal end of the larval filament. It is the

second maxillæ and not the maxillipeds to which the filament is transferred. The tips of these second maxillæ do not unite to form the button, they are simply stuck to the proximal end of the filament (fig. 44). Although the claws subsequently disappear and the maxillæ apparently fuse, sections show that each really preserves its identity; that is, the fusion is only apparent and not real.

10. In the second copepodid stage sex distinction is possible. The body of the male is strongly constricted into a waist between the head and thorax; the second maxillæ are stout and no longer than the maxillipeds. The body of the female is long and slender and only slightly constricted; the second maxillæ are much longer than the maxillipeds and slender.

11. In this second copepodid stage the digestive tract of the male is not yet opened at the anus, but it is completed in the female. The sexual organs have begun to develop and the large maxillary and maxillipedal glands are forming. The nervous system is concentrated in the infra-œsophageal ganglion, and there are no ganglia on the ventral nerve cord.

12. This second copepodid larva molts into the adult form. We have here then a marked concentration of development, the nauplius and metanauplius stages passed inside the egg and so fused as to be indistinguishable, the first copepodid the only free-swimming stage, and of very short duration (twenty-four to forty-eight hours), just long enough to find a host, the second copepodid stage showing the beginning of all the organs and molting directly into the adult.

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## EXPLANATION OF PLATES.

### Plate 29.

*Formation and segmentation of the egg.*

Fig. 1. Section of oviduct, full of developing eggs, each with a cell filament attached to one pole; *b*, coiled filament.

2. A single, fully developed oöcyte, with attached cell filament, more highly magnified.
3. Division and migration of the germinal vesicle.
4. The first cell of the germinal layer.
5. The first two cells of the germinal layer.
6. The germ layer forming into a blastoderm disk.
7. Blastoderm disk half surrounding the egg.
8. Completion of blastoderm disk and formation of blastoderm cuticle.
9. Longitudinal section of nauplius larva, showing the beginnings of the appendages; *a′* and *a″*, first and second antennæ; *fg.*, frontal gland; *m*, muscles which are to operate the swimming legs; *md.*, mandible; *mx′*, and *mx″*., first and second maxillæ; *mxp.*, maxilliped; *s′* and *s″*, first and second swimming legs.

### Plate 30.

*Development within the egg, nauplius stages.*

Fig. 10. Dorsal view of nauplius larva, freed from the egg membranes.

11. Ventral view of same.
12. Longitudinal horizontal section through a nauplius larva; *a′* and *a″*, first and second antennæ; *ab*, fused genital segment and abdomen; *fg.*, frontal gland; *lb.*, labrum; *md.*, mandible; *w.*, body wall.
13. A section through the peg end or origin of the attachment filament; *af.*, attachment filament; *fg.*, frontal gland; *p.*, peg.
14. Attachment filament in two stages of growth; *a*, curved; *b*, cork-screw shaped.
15. Side view of fully developed metanauplius within the egg.

### Plate 31.

*Development within the egg, metanauplius stage.*

Fig. 16. Ventral view of metanauplius larva, freed from the egg membranes; *a′* and *a″*, first and second antennæ; *md.*, mandible; *mx′*. and *mx″*., first and second maxillæ; *mxp.*, maxilliped.

17. First and second antennæ enlarged.
18. A diagonal longitudinal section through the body of a fully developed metanauplius coiled inside the egg membranes; lettering as in fig. 9 with these additions: *af.*, attachment filament; *e*, rudimentary eye; *lb.*, labrum; *ms.*, mesenteron epithelium forming over ventral surface of the stomach; *pg.*, paragnath.
19. The second maxillæ and the attachment filament of the second copepodid stage, showing the claws of the maxillæ imbedded in the end of the filament.
20. A single maxilla with the claw pried out of the filament.
21. Tip of maxilla and claw enlarged.

## PLATE 32.

*The free-swimming larva, first copepodid stage.*

Fig. 22. Ventral view of first copepodid larva, showing appendages.
23. Dorsal view of same.

## PLATE 33.

*The second copepodid stage.*

Fig. 24. Side view of male larva.
25. Dorsal view of same.
26. Side view of antennæ and mouth parts.
27. Side view of female larva.
28. Dorsal view of same.
29. A first antenna with only two joints and two terminal setæ.
30. Second antenna.
31. Mouth-tube, mandible, and first maxilla.

## PLATE 34.

*Longitudinal sections of male and female second copepodid larvæ.*

The lettering of the digestive, excretory, and nervous systems applies equally to both figures; that of the reproductive organs is of necessity peculiar.

Fig. 32. The male: *a′*., nerve to first antenna; *dm*., dorso-ventral muscles; *fg*., frontal gland; *gc*., gland cells; *i*, intestine; *iog*, infra-œsophageal ganglion; *k*, cellular mass or plug, Claus' "Körnchenhaufen;" *lb*., nerve to labium; *lm*., longitudinal muscles; *md*., nerve to mandible; *mpg*., maxillipedal gland, median center, the lateral center being at *lc*.; *mx′*., nerve to second maxilla; *mxp*., nerve to maxilliped; *n*, nerve to frontal and maxillipedal excretory glands; *oe*, œsophagus; *p*., plug of secretion of frontal gland filling hole left by removal of attachment filament; *p c*, proctodeum; *t*., testes; *vc*., ventral cord of nervous system.

33. The female: *a*, anus; *a″*, nerve to second antenna; *lm*, longitudinal muscles; *mpg′*, nerve to maxillipedal gland; *mx*, nerve to first maxilla; *o*., ovary; *s*, stomach; *sm*., sphincter muscle; *sog*., supra œsophageal ganglion; *up*., uterine processes of the oviduct.

## PLATE 35.

*Sections showing internal anatomy.*

Fig. 34. Diagonal section through head of metanauplius larva, showing frontal gland and attachment filament; *a*., first antenna; *c*., coils of filament; *e*., rudimentary eye; *fg*., frontal gland; *m*., muscles of swimming legs; *ms*., mushroom enlargement at anterior end of filament; *p*., peg at proximal end of filament; *w*., wall of egg sac.

35. Beginnings of male reproductive organs; *dm*., dorsoventral muscles; *fd*., free portion of the deferent duct; *t*., testes; *vd*., deferent duct.

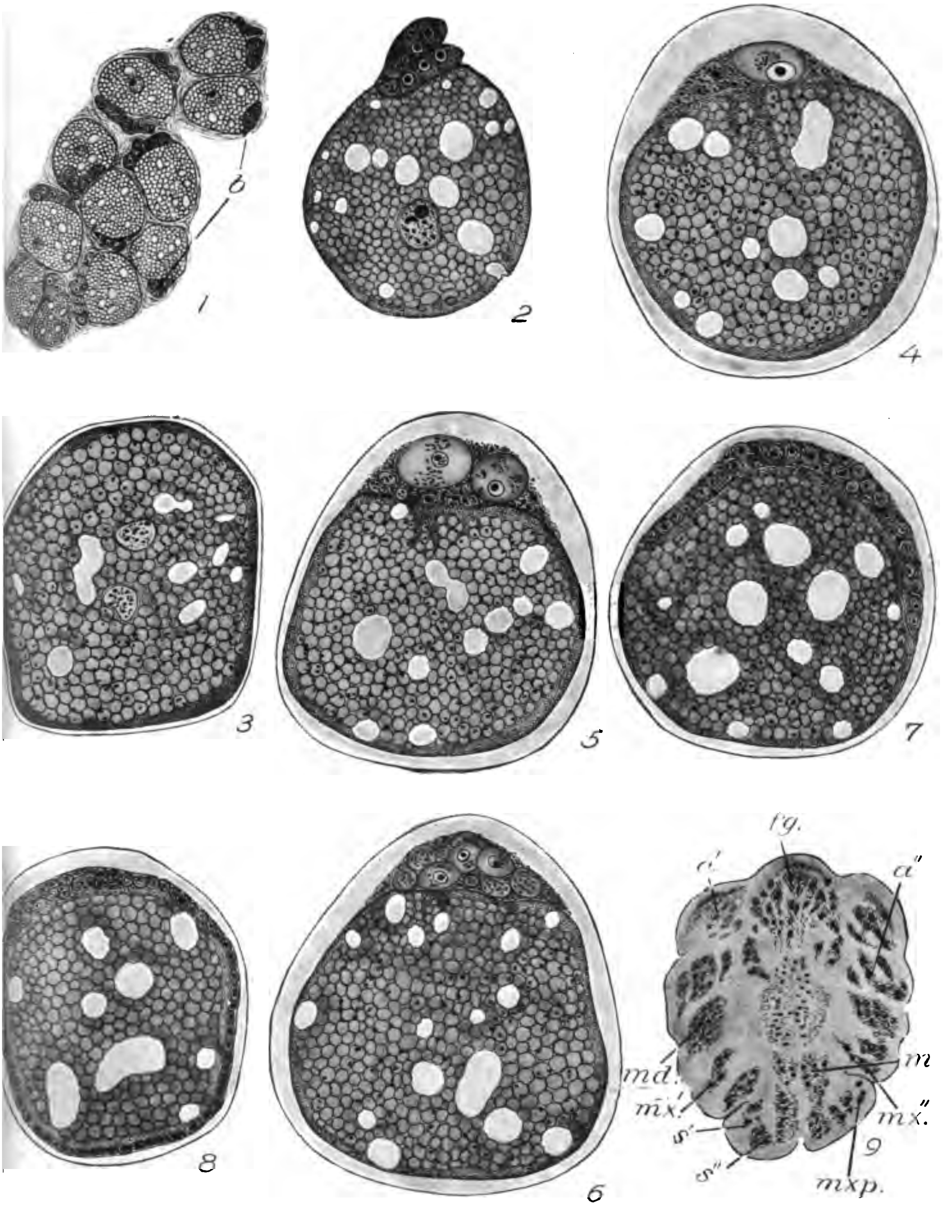
36. Beginnings of female reproductive organs; *cg*., cement gland; *dm*., dorsoventral muscles; *o*., ovary; *od*., oviduct; *up*., uterine processes.

37. The maxillipedal gland of an adult male; *d*., corkscrew duct; *lc*., lateral center of gland; *mc*., median center; *mpg*., main portion of the gland; *mxg*., maxillary gland; *sm*., supporting muscles.

## PLATE 36.

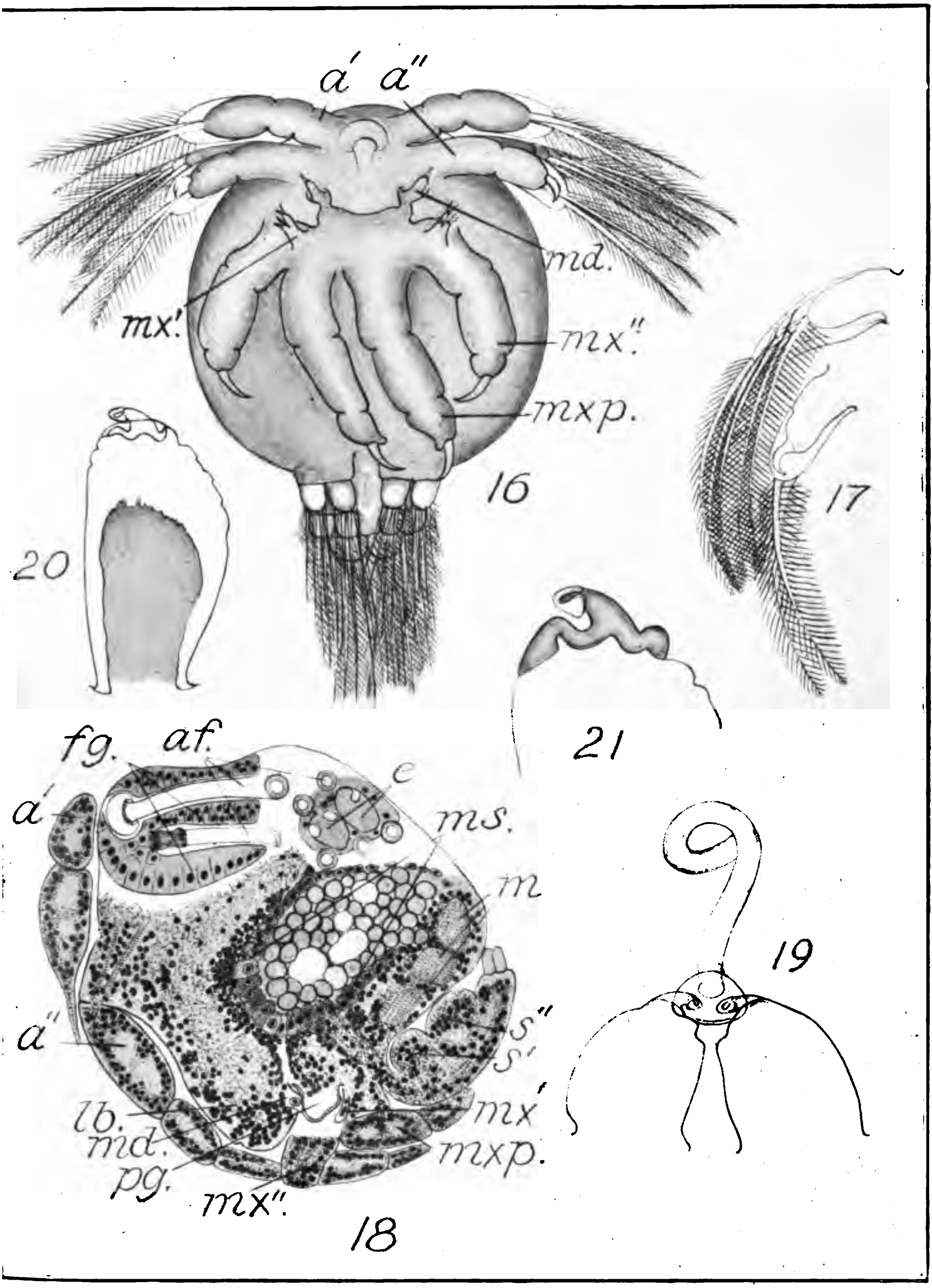
*Young adults of Achtheres ambloplitis.*

Fig. 38. Side view of sexually mature male, attached to spine of gill arch
39. Mouth parts of same.
40. First maxilla.
41. Mandible.
42. Side view of young female.
43. Antennæ and mouth parts of same.
44. Section through the tips of the second maxillæ; *af*., attachment filament; *lm*., longitudinal muscles; *mx*., wall of maxilla.



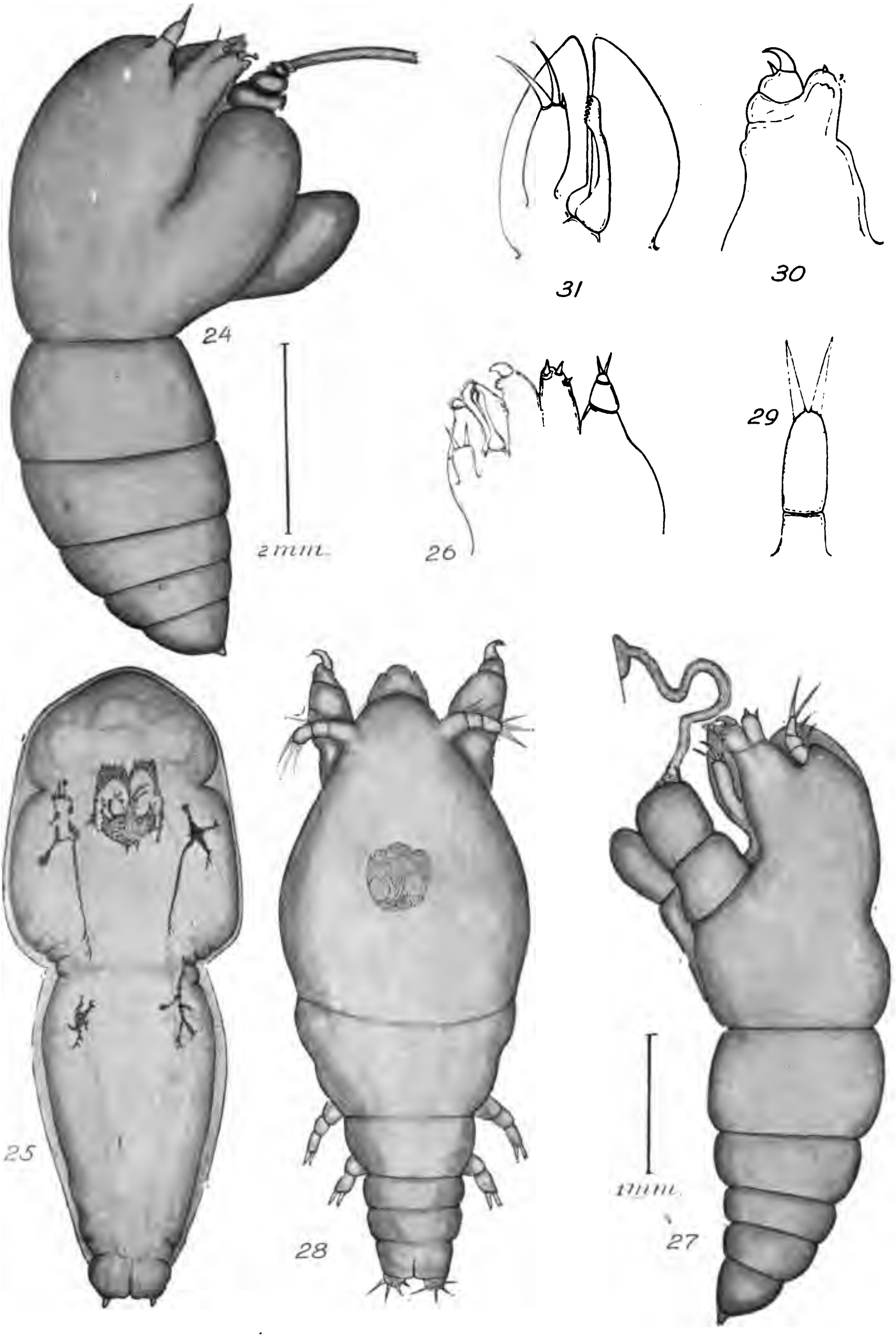
FORMATION AND SEGMENTATION OF THE EGG

FOR EXPLANATION OF PLATE SEE PAGE 225



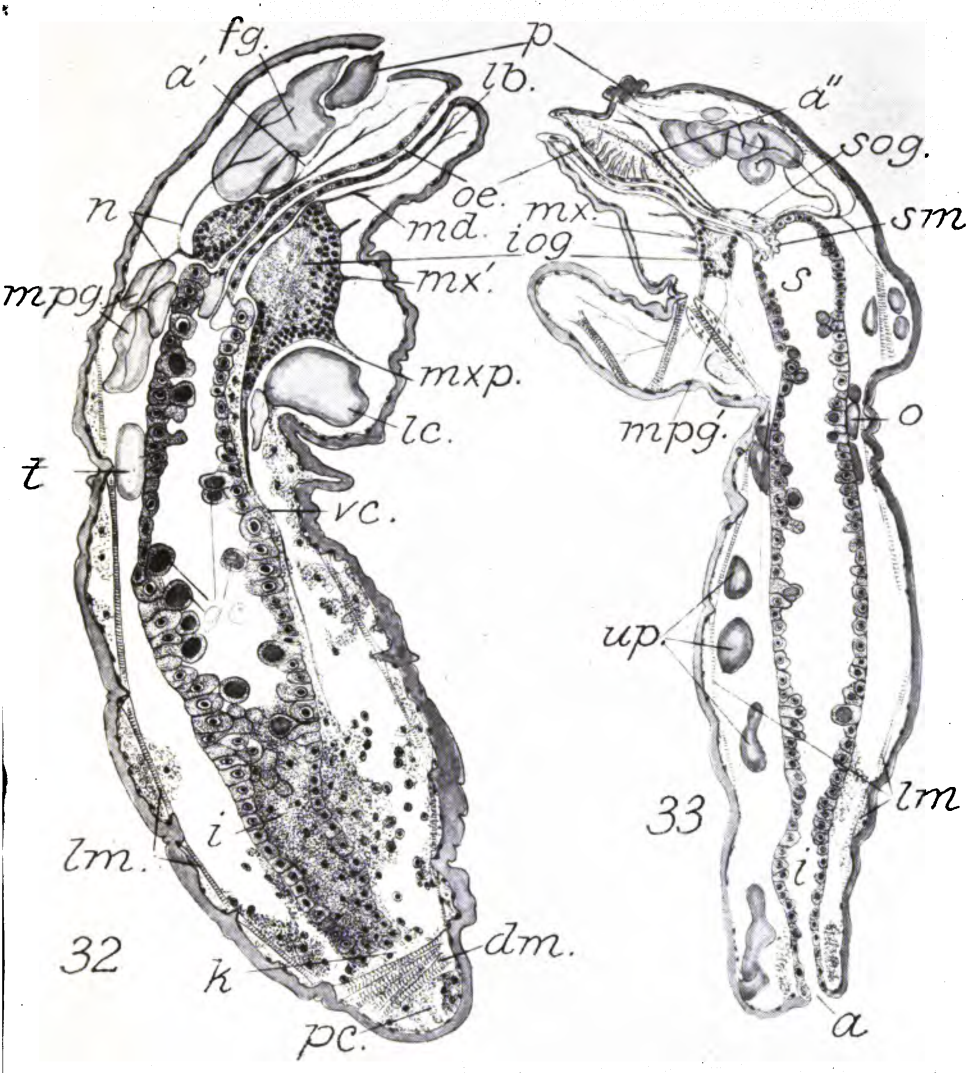
Development Within the Egg, Metanauplius Stage.

For explanation of plate see page 225.



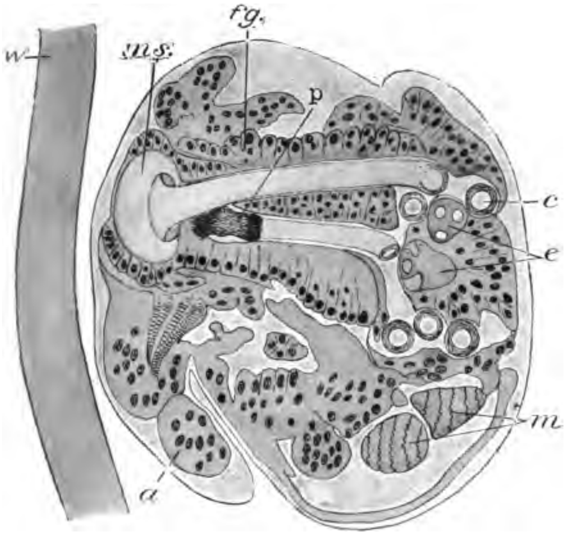
THE SECOND COPEPODID STAGE.

FOR EXPLANATION OF PLATE SEE PAGE 226.

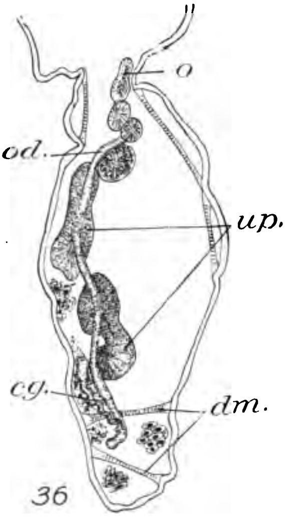


Longitudinal Sections of Male and Female Second Copepodid Larvæ.

For explanation of plate see page 226.



34



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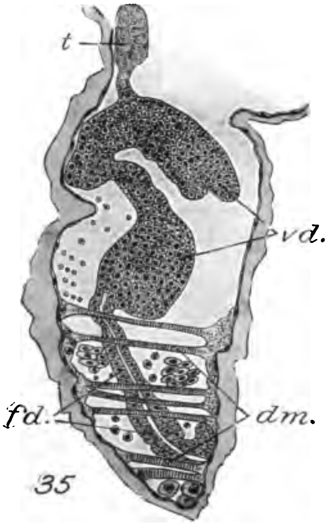

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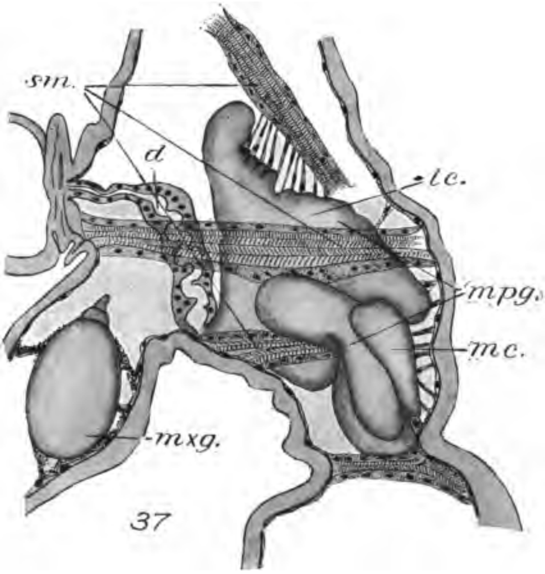

37

SECTIONS SHOWING INTERNAL ANATOMY.

FOR EXPLANATION OF PLATE SEE PAGE 226.